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# Larvivorous fish for preventing malaria transmission (Review)

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[Intervention Review]

# Larvivorous fish for preventing malaria transmission

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## ABSTRACT

### Background

Adult female *Anopheles* mosquitoes can transmit *Plasmodium* parasites that cause malaria. Some fish species eat mosquito larvae and pupae. In disease control policy documents, the World Health Organization (WHO) includes biological control of malaria vectors by stocking ponds, rivers, and water collections near where people live with larvivorous fish to reduce *Plasmodium* parasite transmission. In the past, the Global Fund has financed larvivorous fish programmes in some countries, and, with increasing efforts in eradication of malaria, policymakers may return to this option. Therefore, we assessed the evidence base for larvivorous fish programmes in malaria control.

### Objectives

To evaluate whether introducing larvivorous fish to anopheline larval habitats impacts *Plasmodium* parasite transmission. We also sought to summarize studies that evaluated whether introducing larvivorous fish influences the density and presence of *Anopheles* larvae and pupae in water sources.

### Search methods

We searched the Cochrane Infectious Diseases Group Specialized Register; the Cochrane Central Register of Controlled Trials (CENTRAL), published in the Cochrane Library; MEDLINE (PubMed); Embase (Ovid); CABS Abstracts; LILACS; and the *meta*Register of Controlled Trials (*m*RCT) up to 6 July 2017. We checked the reference lists of all studies identified by the search. We examined references listed in review articles and previously compiled bibliographies to look for eligible studies. Also we contacted researchers in the field and the authors of studies that met the inclusion criteria for additional information regarding potential studies for inclusion and ongoing studies. This is an update of a Cochrane Review published in 2013.

### Selection criteria

Randomized controlled trials (RCTs) and non-RCTs, including controlled before-and-after studies, controlled time series, and controlled interrupted time series studies from malaria-endemic regions that introduced fish as a larvicide and reported on malaria in the community or the density of the adult anopheline population. In the absence of direct evidence of an effect on transmission, we performed a secondary analysis on studies that evaluated the effect of introducing larvivorous fish on the density or presence of immature anopheline

mosquitoes (larvae and pupae forms) in water sources to determine whether this intervention has any potential that may justify further research in the control of malaria vectors.

**Data collection and analysis**

Two review authors independently screened each article by title and abstract, and examined potentially relevant studies for inclusion using an eligibility form. At least two review authors independently extracted data and assessed risk of bias of included studies. If relevant data were unclear or were not reported, we contacted the study authors for clarification. We presented data in tables, and we summarized studies that evaluated the effects of introducing fish on anopheline immature density or presence, or both. We used the GRADE approach to summarize the certainty of the evidence. We also examined whether the included studies reported any possible adverse impact of introducing larvivorous fish on non-target native species.

**Main results**

We identified no studies that reported the effects of introducing larvivorous fish on the primary outcomes of this review: malaria infection in nearby communities, entomological inoculation rate, or on adult *Anopheles* density.

For the secondary analysis, we examined the effects of introducing larvivorous fish on the density and presence of anopheline larvae and pupae in community water sources, and found 15 small studies with a follow-up period between 22 days and five years. These studies were undertaken in Sri Lanka (two studies), India (three studies), Ethiopia (one study), Kenya (two studies), Sudan (one study), Grande Comore Island (one study), Korea (two studies), Indonesia (one study), and Tajikistan (two studies). These studies were conducted in a variety of settings, including localized water bodies (such as wells, domestic water containers, fishponds, and pools (seven studies); riverbed pools below dams (two studies)); rice field plots (five studies); and water canals (two studies). All included studies were at high risk of bias. The research was insufficient to determine whether larvivorous fish reduce the density of *Anopheles* larvae and pupae (12 studies, unpooled data, *very low certainty evidence*). Some studies with high stocking levels of fish seemed to arrest the increase in immature anopheline populations, or to reduce the number of immature anopheline mosquitoes, compared with controls. However, this finding was not consistent, and in studies that showed a decrease in immature anopheline populations, the effect was not always consistently sustained. In contrast, some studies reported larvivorous fish reduced the number of water sources with*Anopheles* larvae and pupae (five studies, unpooled data, *low certainty evidence*).

None of the included studies reported effects of larvivorous fish on local native fish populations or other species.

**Authors' conclusions**

We do not know whether introducing larvivorous fish reduces malaria transmission or the density of adult anopheline mosquito populations.

In research studies that examined the effects on immature anopheline stages of introducing fish to potential malaria vector larval habitats, high stocking levels of fish may reduce the density or presence of immature anopheline mosquitoes in the short term. We do not know whether this translates into impact on malaria transmission. Our interpretation of the current evidence is that countries should not invest in fish stocking as a stand alone or supplementary larval control measure in any malaria transmission areas outside the context of research using carefully controlled field studies or quasi-experimental designs. Such research should examine the effects on native fish and other non-target species.

## PLAIN LANGUAGE SUMMARY

**Fish that feed on mosquito larvae for preventing malaria transmission**

**What is the aim of this review?**

Adult female *Anopheles* mosquitoes transmit the *Plasmodium* parasites that cause malaria. The aim of this Cochrane Review was to evaluate whether introducing fish that eat mosquito larvae and pupae (early life stages of mosquitoes) into water sources near where people live will decrease the adult *Anopheles* mosquito population and thus the number of people infected with *Plasmodium* parasites.

**Key messages**

We do not know if introducing fish that eat mosquito larvae and pupae has an impact on the number of people with malaria or on the adult *Anopheles* mosquito population.

**What was studied in the review?**

The review authors examined the available research that evaluated introducing fish that eat larvae ('larvivorous') to *Anopheles* mosquito larval habitats in areas where malaria was common. Fifteen small studies looked at the effects of larvivorous fish on *Anopheles* larvae and pupae in different larval habitats, including localized water bodies (such as wells, domestic water containers, fishponds, and pools; seven studies), riverbed pools below dams (two studies), rice field plots (four studies), and water canals (two studies). These studies were undertaken in Sri Lanka (two studies), India (three studies), Ethiopia (one study), Kenya (two studies), Sudan (one study), Grande Comore Island (one study), Korea (two studies), Indonesia (one study), and Tajikistan (two studies). This is an update of a 2013 Cochrane Review and includes some older unpublished studies from Tajikistan and a new trial from India.

**What are the main results of the review?**

In our main analysis, we found no studies that looked at the effects of larvivorous fish on adult *Anopheles* mosquito populations or on the number of people infected with *Plasmodium* parasites. In our analysis exploring the effect of fish introduction on the number of *Anopheles* larvae and pupae in water collections, these studies produced inconsistent results on immature mosquito density (12 studies, unpooled data, *very low certainty evidence*). Some studies that measured the number of water sources with*Anopheles* larvae and pupae reported a reduction in the number of sites with *Anopheles* larvae and pupae after introducing fish (five studies, unpooled data, *low certainty evidence*). None of the included studies examined the effects of introducing larvivorous fish on other native species present, but these studies were not designed to do this. All included studies were at high risk of bias.

Before much is invested in this intervention, we need better research to determine the effect of introducing larvivorous fish on the number of people infected with malaria, and on adult *Anopheles* populations. Researchers need to use robust controlled designs with an adequate number of sites. In addition, researchers should explore the potential harms from introducing these fish on native fish and other non-*Anopheles* species.

**How up-to-date is this review?**

The review authors searched for studies published up to 6 July 2017.

## SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [Explanation]

**Larvivorous fish for preventing malaria transmission**

**Patient or population:** people living in malaria-endemic areas
**Settings:** malaria-endemic areas
**Intervention:** larvivorous fish
**Control:** no larvivorous fish

| Outcomes | Illustrative comparative risks* (95% CI) | | Relative effect (95% CI) | Number of studies | Certainty of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| | Assumed risk | Corresponding risk | | | | |
| | Control | Larvivorous fish | | | | |
| **Effects on malaria transmission** | | | | | | |
| Clinical malaria (incidence) | - | - | - | 0 | - | No studies |
| Entomological inoculation rate | - | - | - | 0 | - | No studies |
| Density of adult malaria vectors | - | - | - | 0 | - | No studies |
| **Effects on larvae at potential mosquito larval sites** | | | | | | |
| Density of immature vector stages in water bodies<br>*Quasi-experimental studies* | - | - | Not pooled. Variable effects reported. | 12 | ⊕◯◯◯<br>**Very low**[1–9] | No clear evidence whether or not larvivorous fish reduce the density of immature anopheline mosquitoes in water bodies |

| | | | | | |
|---|---|---|---|---|---|
| Larval sites positive for immature vector stages<br>*Quasi-experimental studies* | - | - | Not pooled<br>Positive effects reported | 5 | ⊕⊕◯◯<br>**Low**[1,2,10–12] | Larvivorous fish may reduce the number of larval sites positive for immature anopheline mosquitoes |

*The basis for the **assumed risk** (for example, the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
**Abbreviations: CI:** confidence interval.

GRADE Working Group grades of evidence.
**High certainty:** further research is very unlikely to change our confidence in the estimate of effect.
**Moderate certainty:** further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
**Low certainty:** further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
**Very low certainty:** we are very uncertain about the estimate.

[1]Downgraded by two: the included studies were non-randomized controlled trials.
[2]No serious risk of bias: all studies had additional problems such as a small number of sites sampled, but these were not deemed adequate to further downgrade the evidence.
[3]No serious inconsistency: seven studies found substantial reductions in immature vector density at the intervention sites (Haq 2013; Howard 2007; Kim 2002; RTDC 2008; Sitaraman 1976; Yu 1989; Zvantsov 2008). For Zvantsov 2008, the effect of *Poecilia reticulata* was not sustained in one site even after reintroduction of fish.
[4]No serious indirectness: these seven studies introduced larvivorous fish into household water sources in India (Haq 2013; Sitaraman 1976), ponds in Kenya (Howard 2007), and rice fields in Korea (Kim 2002; Yu 1989) and Tajikistan (RTDC 2008; Zvantsov 2008). The longest follow-up was in India and still showed benefit at 12 months (Haq 2013). In one study from India, the duration of effect seemed to be influenced by the number of fish introduced (Sitaraman 1976). For Zvantsov 2008, the effect of *P. reticulata* was not sustained in one site even after reintroduction of fish.
[5]No serious imprecision: although statistical significance was not reported, the effects in some studies appeared large (Haq 2013; Howard 2007; Kim 2002; RTDC 2008; Sitaraman 1976; Yu 1989; Zvantsov 2008).
[6]Downgraded by one for inconsistency: effects were variable. Large effects in water canals in Sudan (Mahmoud 1985), but only until nine months' post-intervention. Effects on immature vector populations in Central Java were dependent on vector species (Nalim 1988). No effect in ponds in Kenya stocked once with fish or restocked every two weeks with fish at follow-up (13 weeks). Some effect in water canals in Kenya restocked with fish every two weeks at follow-up (13 weeks) (Imbahale 2011a).
[7]No serious indirectness: these three studies introduced larvivorous fish into ponds in Kenya (Imbahale 2011a), ponds in Sudan (Mahmoud 1985), and rice fields in Central Java (Nalim 1988). The longest follow-up was in Central Java (six years) but showed different effects upon different vector species. In one study from Kenya, the effect seemed to be influenced by the type of site, as an effect was observed in water canal sites but not in pond sites.

[8]Downgraded by one for inconsistency: effects were variable. In one study, no major difference between control and intervention groups was detected at final follow-up (120 days), but area under the curve suggested more rapid decline in larvae in intervention group (Kusumawathie 2008a). In one study, control and intervention groups were not matched at baseline (intervention group higher). However, substantively lower values were detected in the intervention arm at follow-up (one year) (Kusumawathie 2008b).

[9]No serious indirectness: two studies introduced larvivorous fish into riverbed pools below dams in Sri Lanka (Kusumawathie 2008a; Kusumawathie 2008b). The longest follow-up still showed benefit at one year post-intervention in one study. However, control and intervention groups were not matched at baseline (intervention group higher) in all studies.

[10]No serious indirectness: study introduced larvivorous fish into household water sources in Ethiopia (Fletcher 1992). Benefit was still shown at follow-up (one year).

[11]No serious inconsistency: both studies found substantial reductions in immature vector density at the intervention sites (Menon 1978; Sabatinelli 1991).

[12]No serious indirectness: these two studies introduced larvivorous fish into household water sources in Grande Comore Island (Sabatinelli 1991) and India (Menon 1978). The longest follow-up was in Grande Comore Island and still showed benefit at one year post-intervention.

# BACKGROUND

## Description of the condition

Malaria is the most common vector-borne disease worldwide and is endemic in 91 countries. At the start of 2016, almost half the world's population was at risk of malaria. The World Health Organization (WHO) reported an estimated 212 million new cases of malaria worldwide (range 148 million to 304 million) and 429,000 deaths (range 235,000 to 639,000) from malaria in 2015. People living in sub-Saharan Africa continue to be at highest risk of contracting the disease; the WHO African Region accounted for an estimated 90% of malaria cases and 92% of malaria deaths in 2015 (WHO 2016). *Plasmodium* spp. parasites cause malaria in humans and are transmitted by female mosquitoes of the genus *Anopheles*. Of approximately 430 *Anopheles* species, between 30 and 50 species act as dominant vectors.

The main strategies for preventing and controlling malaria include the following:

- prevention through vector control, mainly using long-lasting insecticidal nets (LLINs) (Gleave 2017; Lengeler 2004), or indoor residual spraying (IRS) (Pryce 2017; Tanser 2007), or both (Choi 2017a);
- early diagnosis and effective treatment of people with malaria (Sinclair 2009; Sinclair 2011; Sinclair 2012), chemoprevention in high-risk groups (Garner 2006), and seasonal chemoprophylaxis (Meremikwu 2012).

LLINs and IRS were developed against the most effective vectors, which share the attributes of feeding late at night and being anthropophilic (preferring to feed on humans), endophagic (preferring to feed indoors), and endophilic (preferring to rest indoors) (Lengeler 2004; Tanser 2007). However, many vectors, particularly in Asia and South America (but also in Africa), prefer animals to humans for their blood meals (are zoophilic) or feed early in the evening or outside of houses, where they will be less likely to encounter LLINs or IRS. The two main vector control strategies may be less effective in regions where vectors have these behavioural attributes. These factors have led some agencies and governments to propose other strategies for vector control, and interest in larviciding as a potential means of malaria control has been renewed (Ejov 2014; NVBDCP 2017; WHO-EURO 2006; WHO-GMP 2012; WHO 2017).

## Description of the intervention

Larviciding attempts to control malaria by seeking to reduce the size of the immature vector population. Strategies include the following.

1. Permanently or temporarily reducing the availability of larval habitats (habitat modification and habitat manipulation (Tusting 2013).
2. Adding to standing water microbial or chemical substances that kill or inhibit the development of aquatic immature mosquito stages (Choi 2017b; Lacey 1990; Tusting 2013).
3. Providing biological control by introducing fish (Pyke 2008; Walton 2007), frogs (Raghavendra 2008), or invertebrate predators (such as dragonfly nymphs).

A separate Cochrane Review summarizes larviciding for strategies (1) and (2) (Tusting 2013). The review authors examined cluster-randomized controlled trials, controlled before-and-after trials with at least one year of baseline data, and randomized cross-over trials that compared larval source management (LSM) with no LSM for malaria control. The review authors found some large effects in some studies but not in others. They concluded that when larval habitats were not too extensive, and when a sufficient proportion of these habitats could be targeted, LSM probably reduces the number of people who develop malaria and probably reduces the proportion of the population infected with the *Plasmodium* parasite at any one time (*moderate certainty evidence*). In the included studies, the intervention appeared to be effective in reducing malaria transmission in a range of countries where larviciding was implemented at a wide variety of sites. In one study from The Gambia, where mosquito larval habitats were large swamps and rice paddies, spraying of swamps with larvicide by ground teams did not lead to any benefit. A separate Cochrane Review, which focuses on larviciding alone, is in preparation (Choi 2017b). In this review, we evaluated the most common strategy for biological control: the use of fish that attack mosquito larvae and pupae.

The potential of the larvivorous fish *Gambusia* (*Gambusia affinis* and *Gambusia holbrooki*; Pyke 2005) to ingest large numbers of mosquito larvae led to a series of laboratory-based studies on mosquito larval prey preferences and the optimization of systems to propagate these fish. Subsequently, field evaluations of *Gambusia* were undertaken to assess their impact on larval prevalence and density in mosquito larval habitats.*G. affinis* and *G. holbrooki* are native to the south-eastern USA but have been transported and released in multiple countries globally, so that these species are now collectively the most widely geographically dispersed freshwater fishes in the world (Pyke 2008).

*Gambusia* may adversely affect native fishes and other organisms besides mosquitoes when introduced into new areas. Specialists are now examining the use of native fish species for larval control. Approximately 315 larvivorous fish species belonging to 32 genera under seven families are used for mosquito control, and the family Cyprinodontidae contribute the highest number of genera (15) and species (300) (Goutam 2013). Other promising species for mosquito control belong to the genera *Aphanius*,*Valencia*,*Aplocheilus*,*Oryzias*, *Epiplatys*,*Aphyosemion*,*Roloffia*,*Nothobranchius*,*Pachypanchax*,*Rivulus*,*Fundulus*, and *Cynolebias* (Walton 2007).

## How the intervention might work

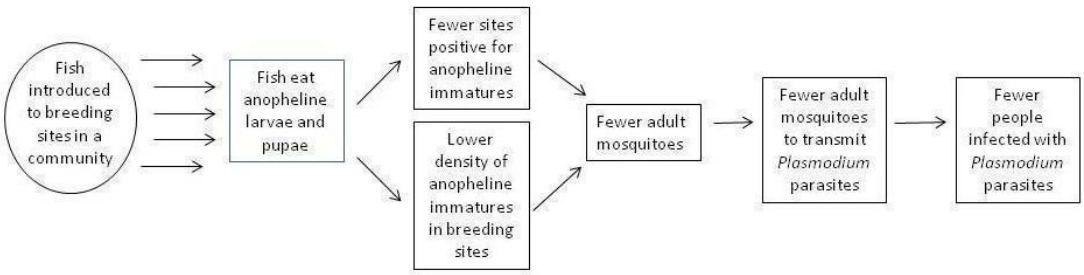
As adult female *Anopheles* mosquitoes transmit malaria, the intensity of transmission is partly dependent on the following:

- whether *Anopheles* are infected with the *Plasmodium* sporozoite stage; and
- how many *Anopheles* feed on humans during the transmission season or year.

The percentage of infected mosquitoes multiplied by the biting rate is a common parameter by which to estimate the force of infection, and is called the entomological inoculation rate (EIR). *Anopheles* mosquitoes lay their eggs in water sources in which they develop into larvae and then pupae. *Anopheles* larvae are found in a wide range of habitats, including fresh- or salt-water marshes; rice fields; mangrove swamps; edges of streams and rivers; grassy ditches; and small, temporary rain pools. Most species prefer clean, unpolluted water. Some mosquitoes may prefer specific sites in which to lay eggs, whilst others use a wide variety of larval habitats (such as temporary ground water pools, including footprints and ditches, as well as more permanent water sources, such as swamps and wells). The abundance of adult mosquitoes depends on a variety of factors, including the number and size of suitable oviposition sites and the density of the immature mosquito stages at these sites. Several other ecological and environmental factors may influence the adult anopheline population, such as temperature, rainfall patterns, and availability of bloodmeal sources.

The larger the mosquito population, the greater the potential number of bites by vectors on humans, unless people take measures to avoid mosquito bites, such as sleeping under an LLIN. For a given sporozoite rate, increases in human-biting rate or mosquito density, or both, will result in higher inoculation rates and greater malaria transmission. If the size of the vector population is limited by interventions that reduce the number of larval habitats or the density of vector larvae per larval habitat, then malaria transmission to humans (with all other factors remaining the same) might potentially be reduced (Figure 1). However, reducing the density of anopheline immature mosquitoes at a larval habitat might have little or no effect on adult numbers because adult numbers may be determined largely or entirely by other factors. Reductions in the density of immature vectors could result in larger, more robust, longer-lived adults through reduced competition between immature *Anopheles* for resources (density-dependent effects), thereby minimizing the potential reduction in malaria transmission. However, Bond 2005 demonstrated that *Anopheles pseudopunctipennis* larvae had significantly prolonged developmental times in the presence of *Poecilia sphenops* fish and emerged as smaller adults. Smaller adult females can have reduced host-seeking responses (Takken 1998), and may produce smaller egg batches (Lyimo 1993).

**Figure 1. Larvivorous fish for preventing malaria transmission: conceptual framework.**




## Why it is important to do this review

The WHO recommendations from 2012 state that antilarval measures are likely to be cost-effective for control of malaria in areas where the larval habitats are limited in number, permanent, and easily found (that is, they are "fixed, finite and findable") (WHO-GMP 2012). The WHO has stated that environmental factors that increase the likelihood that larval control will be effective include a short transmission season, cool temperatures that extend for the duration of the immature stages, and larval habitats that are man-made and homogeneous in nature. In Africa, larviciding is thought to have the best potential to be effective in urban and arid areas and possibly in the East African highlands (WHO-GMP 2012). Indeed, the Cochrane Review of mosquito LSM indicated that the intervention often appeared to impact transmission when implemented in areas where it was feasible to do so (Tusting 2013).

Whether larvivorous fish are an option for LSM is the subject of this Cochrane Review. Since the 1970s, the WHO has promoted the use of larvivorous fish as an environmentally friendly alternative to insecticide-based interventions for malaria control.

A WHO-sponsored interregional conference on malaria control in 1974 reported that "the utilization of larvivorous fish, mainly *Gambusia* or suitable local species, is the only practical measure that can be recommended where applicable, as in lakes, ponds, pools, wells, rice fields" (WHO 1974). A 2001 regional meeting in Kazakhstan recommended that more studies on larger numbers of local larvivorous and phytophagous fish be undertaken in different eco-epidemiological settings in that region, and that the search for effective larvivorous fish should continue (WHO-EURO 2001).

More recently, momentum has gathered in efforts to eliminate malaria, resulting in the 2006-2015 WHO-EURO regional strategy (WHO-EURO 2006) and the 2014-2020 WHO-EURO regional strategy (Ejov 2014), which includes larval control by introduction of larvivorous fish preferentially over other forms of larviciding. However, in its current framework for malaria elimination, the WHO does not include larvivorous fish among the recommended vector control strategies for elimination of malaria (WHO 2017).

WHO recommendations for larviciding as a general strategy are guarded and conditional, but the use of fish is often included in listings of options, alongside clearly established effective measures such as LLINs. For example, the WHO integrated vector management plan to control malaria includes the "effective use of biologically-based agents such as bacterial larvicides and larvivorous fish" (HELI 2005). Fish were one of the traditional means of malaria control in the ex-Soviet Republics of Central Asia, where their use continues (Kondrashin 2017; RTDC 2008; Zvantsov 2008). For example, the Global Fund provided funds for implementation of larvivorous fish against malaria in Tajikistan, although this investment appears modest (UNDP 2013).

Thus, there appear to be differing views on whether introducing larvivorous fish is an effective larvicidal approach; some are strong advocates, whilst others question whether sufficient evidence exists to demonstrate its effectiveness, and whether the strategy can achieve the large reductions in larval numbers required to impact the size of the adult population. In addition, problems are associated with finding and treating all anopheline mosquito larval habitats within a specific area, and some larval habitats may be unsuitable for treatment. Dissemination of larvivorous fish as a control strategy for malaria has the potential for adverse effects on local ecosystems by reducing or eliminating indigenous fish, amphibians, and invertebrates (Walton 2007).

Therefore, we carried out a systematic review of reliable research examining whether evidence shows that this form of larviciding has an impact on malaria. We also sought evidence of the potential to affect transmission, by summarizing studies on the effects of introducing fish on the density and presence of immature anopheline mosquitoes at potential larval habitats. This is an update of a Cochrane Review published in 2013 (Walshe 2013).

# OBJECTIVES

To evaluate whether introducing larvivorous fish to anopheline larval habitats impacts *Plasmodium* parasite transmission. We also sought to summarize studies that evaluated whether introducing larvivorous fish influences the density and presence of *Anopheles* larvae and pupae in water sources.

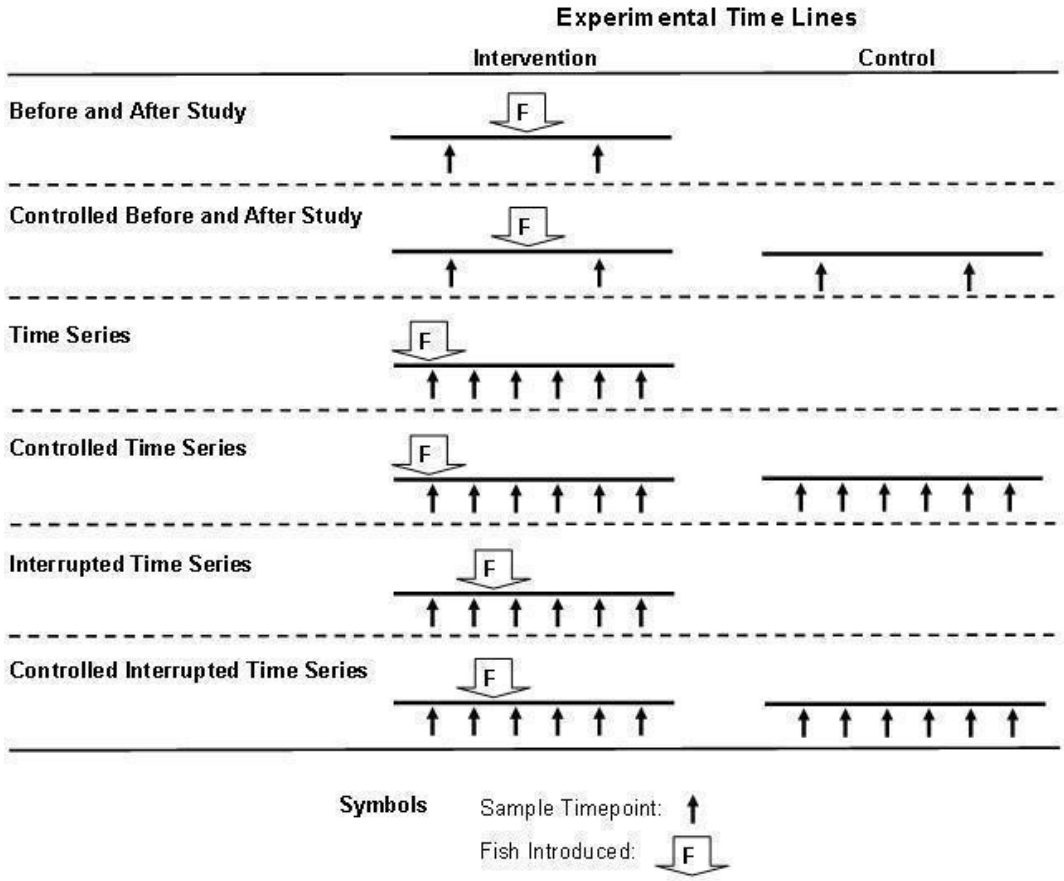
# METHODS

## Criteria for considering studies for this review

### Types of studies

We included randomized controlled trials (RCTs) and non-RCTs, including before-and-after controlled studies, controlled time series, and controlled interrupted time series designs (Figure 2). Comparison groups were geographically defined areas, and thus for RCTs, cluster-randomized designs were used. To be included, intervention and control groups needed to have the following:

**Figure 2. Experimental designs that have been used to attempt to evaluate the impact of fish on the larvae of vectors in malaria-endemic countries. In this figure, we depicted either two or six sample time points (shown by the arrows) as examples. Studies may sample at more time points, or at fewer time points in the case of time series studies.**




- equivalent accompanying antimalarial interventions;
- baseline information;
- contemporaneous data collection;
- same locality (within the same regional area of the country);
- comparable resident populations in relation to ethnic groups, housing, and wealth, based on baseline data provided within the study;
- similar intensities of malaria transmission, based on baseline data provided within the study; and
- sufficient geographic size to minimize masking of the impact of the intervention by immigrating vectors.

In studies of malaria transmission, we specified that intervention and control sites were at least 1 km apart with a human population sample size adequate to detect greater than 25% reduction in *Plasmodium* parasite-positive people.

### Types of participants

Children and adults living in rural and urban malaria-endemic areas.

### Types of interventions

#### Interventions

Introduction of larvivorous fish of any species, either adults or juveniles, into anopheline mosquito larval habitats. This may have been done as a single intervention or as part of a more comprehensive vector control programme that included access to and use of LLINs, IRS, larvicides (including microbial larvicides and insect

growth regulators), polystyrene beads, and environmental management.

Due to seasonal, climatic, and random variations at both immature (larvae and pupae) and adult stages, we included studies that monitored for one or more full years before fish were introduced and those that monitored at one or more time points at least 12 months after fish were introduced into intervention areas. For studies of immature anopheline mosquito populations, we included only studies with a follow-up period longer than three weeks, so that several generations of immature anophelines were monitored.

#### Controls

No larvivorous fish were introduced into control areas. All other vector control measures were the same in intervention and control arms. Thus, for example, we excluded studies that examined introduction of larvivorous fish combined with IRS and those that did not use IRS in the control arm.

### Types of outcome measures

#### Primary outcomes

- Number of confirmed episodes of malaria among community members: defined as malaria infections as laboratory-confirmed cases of malaria (*Plasmodium* parasitaemia detected by microscopy or by rapid diagnostic tests in active or passive case detection).
- Entomological inoculation rate (EIR): defined as the estimated number of bites by infectious mosquitoes per person per unit of time (the product of the number of bites per person per day during the transmission season or per year by vector mosquitoes (the "human-biting rate") and the fraction of vector mosquitoes that are infectious (the "sporozoite rate")).
- Density of adult vector mosquitoes: included measures in which sampling techniques appropriate for these vectors were used, including counting adult anopheline mosquitoes that either landed on exposed body parts of humans acting as bait or were collected resting inside buildings with the use of knockdown spray catches.

#### Secondary outcomes

- Density of immature vector stages at larval sites, as measured by larval dipping (Silver 2008).
- Percentage of larval sites positive for immature anopheline mosquitoes.

In any studies that met the inclusion criteria, we checked whether the study authors reports on native fish populations or other effects on the local ecosystem.

## Search methods for identification of studies

We searched for all relevant studies regardless of language or publication status (published, unpublished, in press, or ongoing).

### Electronic searches

We examined the following databases up to 6 July 2017 using the search terms detailed in Appendix 1: the Cochrane Infectious Diseases Group Specialized Register; the Cochrane Central Register of Controlled Trials (CENTRAL); MEDLINE (PubMed); Embase (Ovid); CABS Abstracts; and LILACS. We searched the *meta*Register of Controlled Trials (*m*RCT) using 'malaria' and 'larvicide* or fish' as search terms; and the literature database of the Armed Forces Pest Management Board using the search terms ('frogs' and 'fish') and 'malaria'.

### Searching other resources

#### Reference lists

We checked the reference lists of all studies identified by the above methods, references listed in review articles (Beltran 1973; Chandra 2008; Pyke 2008; Walker 2007), and previously compiled bibliographies (Gerberich 1968) to identify potential studies.

#### Researchers

We contacted researchers in the field and the authors of studies that met the inclusion criteria for additional information regarding potential studies for inclusion and ongoing studies.

## Data collection and analysis

### Selection of studies

Two review authors screened the abstract of each title obtained from the search for potentially relevant studies. We retrieved the corresponding full articles of these identified studies, and two review authors assessed inclusion by using an eligibility form. We independently screened each search result, assessed each article, and resolved any discrepancies between eligibility results through discussion. If studies did not meet the methods specified, we did not scrutinize further, and if eligibility was unclear, we sought clarification from the study authors.

### Data extraction and management

Two review authors independently extracted data from each study report onto a predesigned data extraction form. We discussed any discrepancies with a third review author.

For the secondary analysis of the effect of introducing larvivorous fish on immature anopheline mosquitoes in water sources, we extracted information on study characteristics and study methods, including setting, comparability between sites, details of the fish intervention, and outcomes, and we examined how study authors measured these. We extracted descriptions of the epidemiology and intensity of transmission from each study, using the terms used by the study authors; co-interventions and whether both control and intervention arms experienced the same co-interventions; and, when study authors presented outcome data in graph or table format, the raw data when possible.

### Design quality

We assessed the study design quality of each included study by examining whether study authors also reported on four specific factors.

- Pupae numbers (as larvivorous fish may preferentially eat particular instars of larvae or pupae) (Bence 1986; Homski 1994; Wurtsbaugh 1980).
- Distance between control and intervention sites.
- Whether other larvivorous species were present.
- Whether vegetation was cleared or removed from the sites.

### Assessment of risk of bias in included studies

For trials that examined the effects of larvivorous fish on malaria transmission, we planned to evaluate the risk of bias using standard Cochrane 'Risk of bias' criteria.

For studies that examined effects on larvae, we assessed risk of bias on the basis of six factors: study design; site selection; site allocation; blinding of assessors; baseline values comparable between sites; and the number of sites. In Table 1, we have shown the exact criteria that we used to assess the risk of bias. Two review authors (DPW and PG for Walshe 2013; DPW and either AAA or TB for this review update) independently assessed the risk of bias for each included study, and resolved any discrepancies by discussion with a third review author.

### Data synthesis

We performed individual critical appraisal of each included study on the possible effects of introduction of larvivorous fish on immature mosquitoes. The large variation in study design, outcomes, and reporting precluded any data synthesis. We tried to draw patterns of effect by grouping studies by habitat as follows.

- Localized water bodies, including wells, domestic water containers, fishponds and man-made pools, and pools in a riverbed below a dam.
- Rice field plots.
- Water canals.

We described each study in a short narrative and presented the outcome results in table format. We reported results at baseline and at pre-specified time points at follow-up, and used the GRADE approach to assess the certainty of the evidence.

## RESULTS

### Description of studies

We have provided descriptions of the included and excluded studies in the Characteristics of included studies and Characteristics of excluded studies tables.

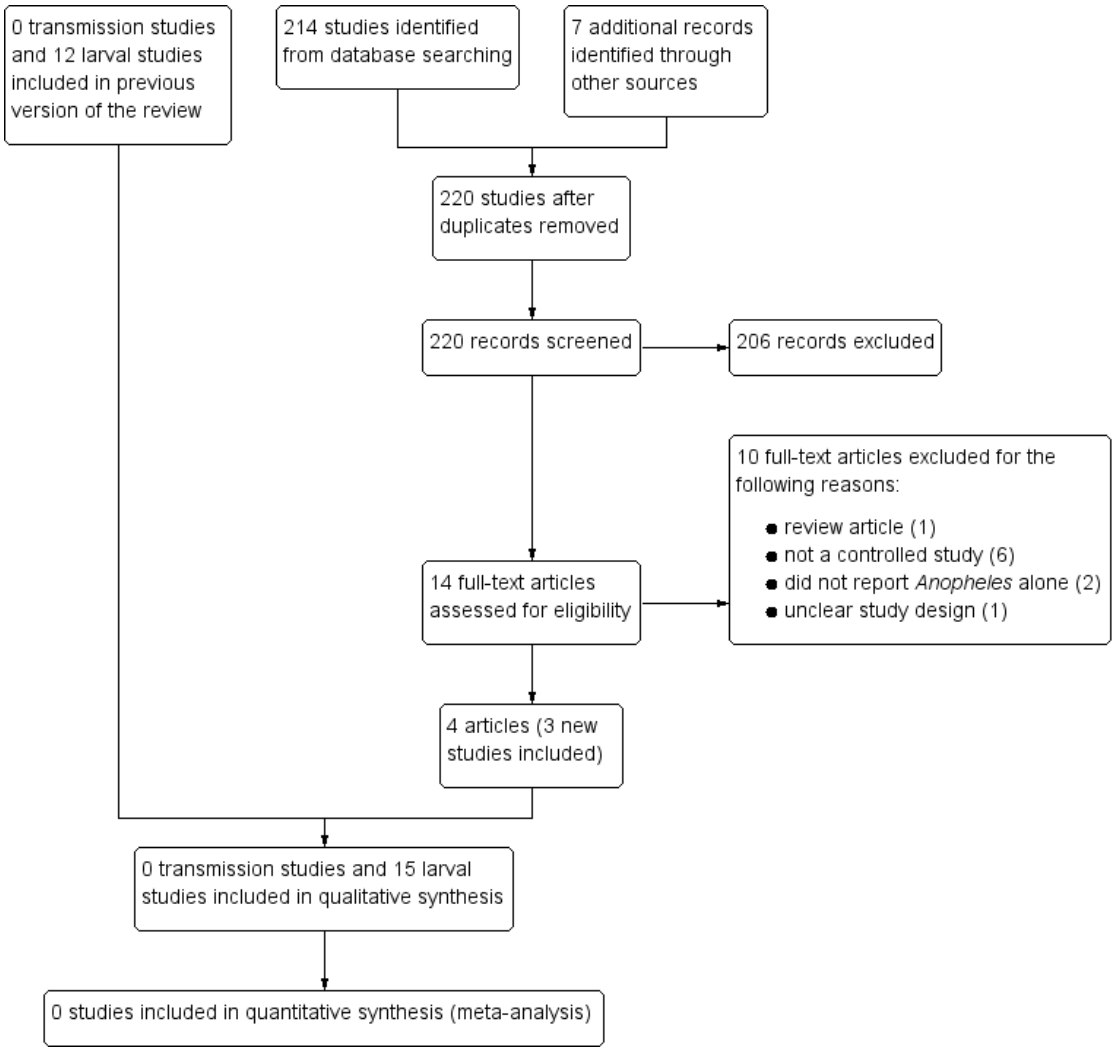
### Results of the search

In the previous version of this Cochrane Review, we identified 1286 titles and abstracts from the electronic search of databases and 12 additional articles after contacting researchers and screening reference lists (Walshe 2013). After we removed duplicates, 915 records remained. Of these, we obtained 117 potentially eligible articles. We identified no studies that fulfilled the selection criteria and reported on primary outcomes. Of the 117 potentially eligible articles, we identified 12 studies that fulfilled the selection criteria for the secondary outcomes only and 105 studies that did not meet the eligibility criteria.

For this review update, we identified 214 titles and abstract from electronic searches of databases and seven additional articles through other sources. There were 220 articles after we removed duplicates. Of these, there were 14 potentially eligible articles. None of these articles fulfilled the inclusion criteria and reported on the primary outcomes. Three new studies (four articles) met the inclusion criteria and reported only on the secondary outcomes (Haq 2013; RTDC 2008; Zvantsov 2008).

We excluded 10 studies after full-text assessment with reasons ( Characteristics of excluded studies table). Figure 3, a study flow diagram, illustrates the study selection process,

**Figure 3. Study flow diagram**




## Included studies

None of the included studies reported on cases of malaria, EIR, or density of adult vector mosquitoes. Thus, there was no direct evidence this intervention impacts malaria transmission. Therefore, our analysis focuses only on the effects of fish stocking on the secondary outcomes: the presence or density of immature mosquitoes in water sources.

### Sites

We summarized the sites by type of water sources stocked, number of sites stocked, and site size (Table 2). Ecological sites included the following.

- Localized water bodies such as wells (Menon 1978; Sitaraman 1976); domestic water containers (Fletcher 1992; Haq 2013; Sabatinelli 1991); fishponds and man-made pools (Howard 2007; Imbahale 2011a); and riverbed pools below dams (Kusumawathie 2008a; Kusumawathie 2008b).
- Rice field plots (Kim 2002; Nalim 1988; RTDC 2008; Yu 1989; Zvantsov 2008).
- Water canals (Imbahale 2011a; Mahmoud 1985).

The number and size of habitat sites chosen by the trial authors varied (see Table 2). For example, Fletcher 1992 introduced fish to 68 habitat sites and maintained 60 habitat sites as controls. Haq 2013 introduced fish to 295 water storage containers in one village, including underground water tanks (127), kothi (big mud

pots) and barrels (167), and no fish to the control village; and monitored 30 containers in the intervention village and 25 in the control village. Menon 1978 stocked fish in 3438 wells and left 317 wells without fish as controls. However, Howard 2007 used two fishponds as intervention sites and one fishpond as a control. Habitat sizes ranged from small, 1 m × 1 m × 1 m man-made ponds (Howard 2007) to 24.8 ha plots of land (Nalim 1988). Notably, Nalim 1988 recorded the number of adult mosquitoes collected in emergence traps, and we used these data to determine the effects of larvivorous fish on the immature mosquito population.

### Design

Of the 15 larval studies that we identified, one was a quasi-RCT (Fletcher 1992), six were controlled interrupted time series (Howard 2007; Kim 2002; Menon 1978; Sabatinelli 1991; Sitaraman 1976; Yu 1989), six were controlled time series (Haq 2013; Imbahale 2011a; Mahmoud 1985; Nalim 1988; RTDC 2008; Zvantsov 2008), and two were controlled before-and-after studies (Kusumawathie 2008a; Kusumawathie 2008b).

Two studies were undertaken in Sri Lanka (Kusumawathie 2008a; Kusumawathie 2008b), three in India (Haq 2013; Menon 1978; Sitaraman 1976), one in Ethiopia (Fletcher 1992), two in Kenya ( Howard 2007; Imbahale 2011a), one in Sudan (Mahmoud 1985), one in Grande Comore Island (Sabatinelli 1991), two in Korea (Kim 2002; Yu 1989), one in Indonesia (Nalim 1988), and two in Tajikistan (RTDC 2008; Zvantsov 2008).

### Intervention

We summarized in Table 3 the key details of the fish intervention provided for each included study.

The study authors used the following fish species in larval studies: *Aphanius dispar* (Fletcher 1992; Haq 2013); *Poecilia reticulata* (Kusumawathie 2008a; Kusumawathie 2008b; Nalim 1988; Sabatinelli 1991; Sitaraman 1976; Zvantsov 2008); *Cyprinus carpio* (Nalim 1988); *G. affinis* (Imbahale 2011a; Menon 1978; RTDC 2008; Zvantsov 2008); *G. holbrooki* (Mahmoud 1985); *Aplocheilus blockii* (Menon 1978); *Aplocheilus latipes* (Kim 2002; Yu 1989); *Aphyocypris chinensis* (Kim 2002); *Oreochromis niloticus* (formerly *Tilapia nilotica*) (Howard 2007); and *Tilapia mossambicus niloticus* (Kim 2002; Yu 1989). Two studies also used the herbivorous species *T. m. niloticus* to control aquatic weeds, but they did not directly use this fish species for immature mosquito predation (Kim 2002; Yu 1989). Seven studies introduced fish species that were indigenous to the area (Fletcher 1992; Haq 2013; Howard 2007; Kim 2002; Menon 1978 (*A. blockii* only); Nalim 1988 (*C. carpio* only); Yu 1989 (*A. latipes* only)). Twelve studies used non-indigenous fish species (Imbahale 2011a; Kim 2002 (*T. m. niloticus* only); Kusumawathie 2008a; Kusumawathie 2008b; Mahmoud 1985; Menon 1978; Nalim 1988 (*P. reticulata* only); RTDC 2008; Sabatinelli 1991; Sitaraman 1976; Yu 1989 (*T. m. niloticus* only); Zvantsov 2008).

The number of fish introduced to sites varied, and stocking density depended primarily on the size of the water body treated (Table 3). Twelve studies did not state the size or maturity of the fish introduced (Fletcher 1992; Haq 2013; Kim 2002; Kusumawathie 2008a; Kusumawathie 2008b; Mahmoud 1985; Menon 1978; Nalim 1988; RTDC 2008; Sabatinelli 1991; Sitaraman 1976; Yu 1989). Only three studies reported the size (Imbahale 2011a), or the maturity (Howard 2007; Zvantsov 2008), of the larvivorous fish introduced to the sites. Only two studies reported the sex ratio of fish introduced (Kusumawathie 2008a; Kusumawathie 2008b), but the remaining 13 studies did not. Twelve studies reported the time of year that fish were introduced to the intervention site (Fletcher 1992; Haq 2013; Howard 2007; Imbahale 2011a; Kim 2002; Kusumawathie 2008a; Kusumawathie 2008b; Mahmoud 1985; Menon 1978; Sabatinelli 1991; Yu 1989; Zvantsov 2008), and three studies did not (Nalim 1988; RTDC 2008; Sitaraman 1976). Nine studies monitored fish survival (Fletcher 1992; Haq 2013; Kusumawathie 2008a; Mahmoud 1985; Menon 1978; RTDC 2008; Sabatinelli 1991; Sitaraman 1976; Zvantsov 2008). Six studies performed restocking of fish after regular monitoring of the fish population (Fletcher 1992; Kusumawathie 2008b; Menon 1978), or at pre-specified time points (Imbahale 2011a; Mahmoud 1985; Nalim 1988). For Zvantsov 2008, it was unclear whether *P. reticulata* alone or both *P. reticulata* and *G. affinis* fish species were restocked (Table 3).

### Design quality

We evaluated the following study design quality factors of the included studies and summarized the results in Table 4.

- Pupae numbers reported: larvivorous fish may preferentially eat particular instars of mosquito larvae or pupae (Walker 2007). Therefore, we checked whether studies monitored both larvae and pupae populations. RTDC 2008 and Sitaraman 1976 reported both larvae and pupae numbers. Howard 2007 reported larvae and pupae numbers combined. Fletcher 1992 recorded, but did not report, pupae numbers. Haq 2013 recorded the density of larvae and pupae, but only reported the percentage reduction in larvae (L3 and L4 instar) and pupae. Zvantsov 2008 reported either "younger" or "older" *Anopheles* larvae; the remaining nine studies did not report pupae numbers (Imbahale 2011a; Kim 2002; Kusumawathie 2008a; Kusumawathie 2008b; Mahmoud 1985; Menon 1978; Nalim 1988; Sabatinelli 1991; Yu 1989).
- Distance between sites: two studies had a distance of greater than 1 km between control and intervention sites (Haq 2013; Sabatinelli 1991). Six studies had control and intervention sites that were less than 1 km from each other (Fletcher 1992; Howard 2007; Kim 2002; Kusumawathie 2008a; Yu 1989; Zvantsov 2008). Seven studies did not report the distance between these sites (Imbahale 2011a; Kusumawathie 2008b; Mahmoud 1985; Menon 1978; Nalim 1988; RTDC 2008; Sitaraman 1976).

- Other larvivorous species present: none of the included studies reported whether other larvivorous species were present at the control and intervention sites. Zvantsov 2008 recorded, but did not report, this data. Kim 2002 reported that no other larvivorous fish species were present at the fish intervention site but did not monitor the control site.
- Vegetation cleared: the vegetation coverage can also affect immature mosquito numbers. Twelve studies did not report whether vegetation was cleared at the study sites (Fletcher 1992; Haq 2013; Imbahale 2011a; Kusumawathie 2008a; Kusumawathie 2008b; Mahmoud 1985; Menon 1978; Nalim 1988; RTDC 2008; Sabatinelli 1991; Sitaraman 1976; Zvantsov 2008). Howard 2007 stated that at all sites, vegetation was cleared on a weekly basis. Two studies used the herbivorous fish, *T. m. niloticus*, to clear vegetation. However, Kim 2002 used this fish species at the intervention sites but not at the control sites, and Yu 1989 used this fish species in one treatment arm only.

### Outcomes

Of the 15 included larval studies, 12 studies examined the effects of larvivorous fish on the density of immature *Anopheles* mosquitoes (Haq 2013; Howard 2007; Imbahale 2011a; Kim 2002; Kusumawathie 2008a; Kusumawathie 2008b; Mahmoud 1985; RTDC 2008; Sitaraman 1976; Yu 1989; Zvantsov 2008), or vector adults collected using emergence traps as a measure of larval density (Nalim 1988). Four of these studies were controlled interrupted time series (Howard 2007; Kim 2002; Sitaraman 1976; Yu 1989), six studies were controlled time series (Haq 2013; Imbahale 2011a; Mahmoud 1985; Nalim 1988; RTDC 2008; Zvantsov 2008), and two studies were controlled before-and-after studies (Kusumawathie 2008a; Kusumawathie 2008b). Five studies recorded the percentage of sites positive for larvae of the vector (Fletcher 1992; Kusumawathie 2008a; Kusumawathie 2008b; Menon 1978; Sabatinelli 1991). Of these five studies, one study was a quasi-RCT (Fletcher 1992), two studies were controlled interrupted time series (Menon 1978; Sabatinelli 1991), and two studies were controlled before-and-after studies (Kusumawathie 2008a; Kusumawathie 2008b).

### Excluded studies

In the previous version of this review, Walshe 2013, we excluded 105 studies because they did not meet the eligibility criteria, or they did not report any outcome of interest, or both. We have given the reasons for exclusion in the Characteristics of excluded studies table: *Anopheles* and *Culex* populations were not monitored separately (seven studies); studies were not fish studies (29 studies); no primary outcomes were reported (20 studies); no secondary outcomes were reported (eight studies); multiple interventions were introduced, meaning that the effect of fish alone could not be determined (eight studies); study was laboratory-based, not field-based (four studies); inappropriate study design was applied (54 studies); or the outcome data were already presented in another paper (four studies). In several cases, we excluded a study for more than one reason.

In this review update, we excluded 10 articles after full-text assessment. One was a review article (Chandra 2013), six were not controlled trials (Azevedo-Santos 2016; Brumpt 1928; Coulon 1931; Manimunda 2009; Sunish 2015a; Sunish 2015b), and two studies reported the number of immature mosquitoes in total, but not anopheline mosquitoes alone (Kondrashin 2017; Warbanski 2017). One study had an unclear study design (de Buen 1930).

## Risk of bias in included studies

Table 1 shows the criteria we used to assess the risk of bias in included studies and we have presented our findings in the 'Risk of bias' tables in the Characteristics of included studies section. We have summarized the risk of bias results in Figure 4 and Figure 5.

**Figure 4. 'Risk of bias' summary: review authors' judgements about each 'Risk of bias' item for each included study**

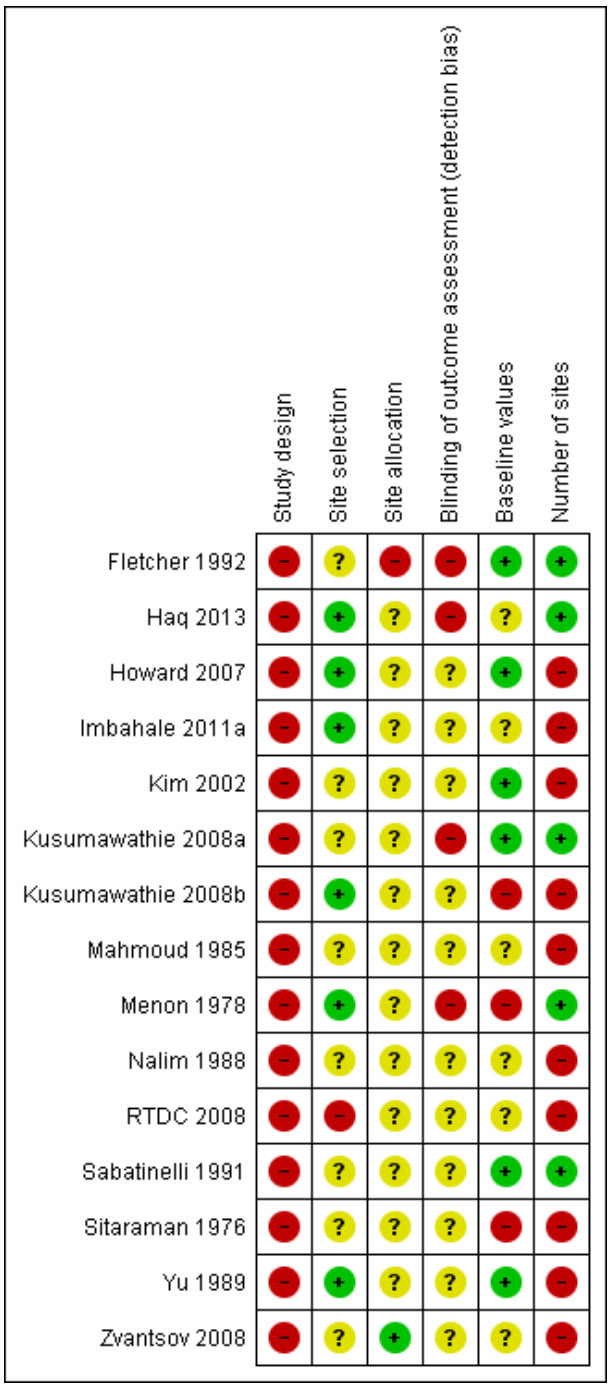
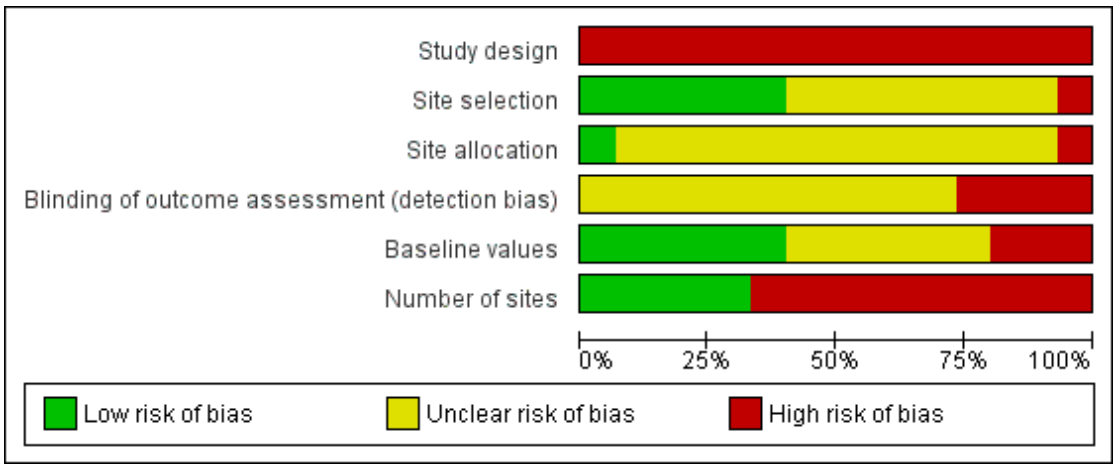


**Figure 5. 'Risk of bias' graph: review authors' judgements about each 'Risk of bias' item presented as percentages across all included studies**




### Study design

None of the studies included randomized comparisons, and therefore all were at high risk of bias.

### Site selection

Eight studies did not state how they selected sites (Fletcher 1992; Kim 2002; Kusumawathie 2008a; Mahmoud 1985; Nalim 1988; Sabatinelli 1991; Sitaraman 1976; Zvantsov 2008), and were at unclear risk of bias. Six studies stated clearly how the sites were selected within the study area and were at low risk of bias (Haq 2013; Howard 2007; Imbahale 2011a; Kusumawathie 2008b; Menon 1978; Yu 1989). One study was at high risk of bias regarding site selection as the intervention and control areas each included one district with malaria cases and one district without malaria cases; the study authors provided no indication regarding how the sites for intervention and control areas were allocated (RTDC 2008).

### Site allocation

Study authors did not give information about how they chose the comparator sites in 13 studies (Haq 2013; Howard 2007; Imbahale 2011a; Kim 2002; Kusumawathie 2008a; Kusumawathie 2008b; Mahmoud 1985; Menon 1978; Nalim 1988; RTDC 2008; Sabatinelli 1991; Sitaraman 1976; Yu 1989), and the studies were at unclear risk of bias. One study was at high risk of bias as sites were allocated to treatment by alternation (Fletcher 1992). One study was at low risk of bias as the study authors stated that allocation of treatment within a checkerboard pattern was random (Zvantsov 2008).

### Blinding of assessors

Study authors did not blind outcome assessors to the intervention in four studies (Fletcher 1992; Haq 2013; Kusumawathie 2008a; Menon 1978), and the studies were at high risk of bias. In the 11 remaining studies, the risk of bias was unclear (Howard 2007; Imbahale 2011a; Kim 2002; Kusumawathie 2008b; Mahmoud 1985; Nalim 1988; RTDC 2008; Sabatinelli 1991; Sitaraman 1976; Yu 1989; Zvantsov 2008).

### Baseline values comparable between sites

In three studies, baseline values before the intervention was introduced were not comparable between control and intervention sites, and the studies were classified as having high risk of bias (Kusumawathie 2008b; Menon 1978; Sitaraman 1976). In Kusumawathie 2008b, baseline values were comparable for two outcomes: mean number of *Anopheles* larvae per 100 dips; and mean monthly percentage of sites positive for *Anopheles* larvae. However, baseline values were not comparable for the two other outcomes: mean monthly number of anopheline larvae per 100 pools; and total number of *Anopheles* larvae; this study was at high risk of bias. Six studies were at unclear risk of bias

(Haq 2013; Imbahale 2011a; Mahmoud 1985; Nalim 1988; RTDC 2008; Zvantsov 2008). Six studies were at low risk of bias (Fletcher 1992; Howard 2007; Kim 2002; Kusumawathie 2008a; Sabatinelli 1991; Yu 1989).

### Number of sites

Five studies were at low risk of bias, as they had an adequate number of sites (20 or more) per group (Fletcher 1992; Haq 2013; Kusumawathie 2008a; Menon 1978; Sabatinelli 1991). We judged eight studies to be at high risk of bias, as four studies may have had an inadequate number of sites (five to < 20) per group (Imbahale 2011a; Mahmoud 1985; Sitaraman 1976; Zvantsov 2008), and six studies probably had an inadequate number of sites (less than five) per group (Howard 2007; Kim 2002; Kusumawathie 2008b; Nalim 1988; RTDC 2008; Yu 1989).

## Effects of interventions

See: **Summary of findings for the main comparison** 'Summary of findings' table 1

### Primary analysis

We identified no studies that reported the primary outcomes (number of confirmed episodes of malaria among community members, EIR, or density of adult vector mosquitoes). Thus, there is no direct evidence that indicates this intervention impacts malaria transmission.

### Secondary analysis

For the secondary analysis of whether introduction of larvivorous fish impacts immature anopheline mosquitoes, all studies were at high risk of bias and provided only indirect evidence of the potential effectiveness of this intervention. As the methods of the included studied varied, we have given a full critical appraisal of each study in Appendix 2 and a summary in Table 5. Fifteen studies met the inclusion criteria, which were conducted in localized water bodies, including wells, domestic water containers, and fishponds and pools (seven studies); pools in a riverbed below a dam (two studies); rice field plots (four studies); or water canals (two studies). Twelve included studies reported on the density of immature anopheline vector stages in water bodies (Haq 2013; Howard 2007; Imbahale 2011a; Kim 2002; Kusumawathie 2008a; Kusumawathie 2008b; Mahmoud 1985; Nalim 1988; RTDC 2008; Sitaraman 1976; Yu 1989; Zvantsov 2008), and five studies reported the number of larval sites positive for immature anopheline vector stages (Fletcher 1992; Kusumawathie 2008a; Kusumawathie 2008b; Menon 1978; Sabatinelli 1991).

Of the 15 included studies, 12 studies reported on the density of immature *Anopheles* mosquito stages in water bodies, and we do not know if larvivorous fish reduce the density (12 studies, unpooled data, *very low certainty evidence*). Some evidence from studies that ranged in size suggested that larvivorous fish could sometimes prevent increases in immature anopheline mosquito densities compared with control sites, and some studies provided evidence of sustained reductions in immature anopheline numbers up to 13 months of follow-up, but these findings were not consistent. Despite stratification by site and careful critical analysis of each individual study, clear patterns were not evident, although stocking density seemed to have some impact on whether introducing larvivorous fish influenced immature anopheline density.

Of the 15 included studies, five studies reported on larval sites positive for immature vector stages. All reported a reduction in the number of sites positive for *Anopheles* immatures or "prevention of an increase" in the number of sites positive for *Anopheles* immatures. Larvivorous fish may reduce the number of larval sites positive for immature anopheline mosquitoes (five studies, unpooled data, *low certainty evidence*).

None of the studies reported on other ecosystem effects, including densities of endogenous fish.

# DISCUSSION

## Summary of main results

We identified no RCTs or quasi-experimental studies that examined the direct impact of larvivorous fish on malaria in people living in malaria-endemic communities; or on outcomes related to transmission, including EIR and the density of adult vector mosquitoes. Therefore, we do not know whether larvivorous fish have an effect on adult anopheline mosquito populations or on malaria transmission in endemic communities.

In addition, we examined whether any evidence suggested that this form of vector control had any potential for an effect on malaria. We examined the effect of larvivorous fish stocking on two secondary outcomes: density of immature vector stages and percentage of larval sites positive for immature anopheline mosquitoes compared with controls. Fifteen small-scale studies met the inclusion criteria of this review and reported on these secondary outcomes only. These studies ranged from three weeks up to five years. They were undertaken in a variety of settings, including localized water bodies (wells, domestic water containers, fishponds or pools, and riverbed pools below dams; nine studies), rice field plots (four studies), and water canals (two studies). Evidence of an effect of larvivorous fish on the density of immature vector stages in water bodies was variable. We do not know from the available evidence whether larvivorous fish reduce the density of immature anopheline stages (12 studies, unpooled data,*very low certainty evidence*). Larvivorous fish may cause a reduction in the percentage of larval sites positive for immature vector stages (five studies, unpooled data, *low certainty evidence*).

Based on the current evidence base and due to the poor quality of the included studies and the absence of any consistent effect, this is not an intervention that could sensibly be used in malaria vector control. Whether these data can guide future research on which larvivorous fish species should be evaluated and which categories of larval sites should be tested is also not entirely clear. Some reports describe almost 100% reduction of the immature *Anopheles* population (Fletcher 1992; Haq 2013; Kusumawathie 2008a; Menon 1978; RTDC 2008; Sitaraman 1976). Effects of the fish intervention on immature anopheline populations were mainly reported in studies that used high stocking densities of fish in localized water bodies with short follow-up periods (less than four months), although one study suggested that increasing larval numbers were inhibited for the 11 months' follow-up in domestic water sources (Fletcher 1992).

Notably, monitoring of the immature mosquito population did not appear to influence decisions regarding implementation, such as fish restocking or increase in fish stocking density, in the included studies. None of the included studies examined the impact, if any, of larvivorous fish introduction on the environment or on native species present apart from the target *Anopheles* mosquito species.

### Overall completeness and applicability of evidence

This review demonstrates that there is currently insufficient evidence regarding whether larviciding with fish impacts cases of human malaria or malaria transmission. In some circumstances, the intervention may lead to a reduction in immature mosquitoes in the water sources stocked with fish. This does not show an effect on malaria transmission but simply shows that the intervention may have a potential benefit worthy of further research.

### Quality of the evidence

We found no evidence for the primary outcome of examining the effects of introducing larvivorous fish on malaria transmission. The certainty of the evidence exploring the larvicidal effect of fish was low or very low, and overall study design was poor.

### Potential biases in the review process

Our search strategy was comprehensive, and it was not limited by language or publication status. Many of the older studies contained anecdotal evidence, and in many studies, fish were combined with other antimalarial interventions in uncontrolled designs, so attribution of an effect was not possible. We contacted study authors where information was missing or unclear.

### Agreements and disagreements with other studies or reviews

There is a Cochrane Review of larvicides that excluded fish (Tusting 2013). This review indicated that larviciding could be effective for preventing malaria transmission, but raised questions about whether it was feasible to undertake this in many areas of Africa.

The current WHO regional strategy for the WHO European Region 2014-2020 recommends the introduction of *Gambusia* fish "into all sites where *Anopheles* breed" in areas with "high receptivity and vulnerability" (Ejov 2014). This endorses the same guidelines of the WHO regional strategy for the WHO European Region 2006-2015, which recommended the use of larvivorous fish "in all existing or potential reservoirs where *Anopheles* species breed with particular attention to rice fields" (WHO-EURO 2006). However, the WHO does not currently recommend this intervention as a vector control strategy for elimination of malaria in its framework for malaria elimination (WHO 2017). The use of larvivorous fish as part of an integrated programme to control malaria has been advocated, subject to further vector biology studies to ensure that the actual vector is targeted (Ghosh 2007). However, further high certainty evidence is required before these recommendations can be supported. Although this Cochrane Review update demonstrated that use of larvivorous fish can cause a significant reduction in the number of immature mosquitoes, particularly in fixed larval sites as opposed to temporary larval sites, a direct correlation between reduction of immature mosquito numbers and reduction of the adult vector population or the number of cases of malaria in people needs to be demonstrated.

## AUTHORS' CONCLUSIONS

### Implications for practice

There is no reliable research evidence that introducing larvivorous fish has any effect on outcomes of transmission of human malaria. Whilst sometimes presented as biologically friendly compared with chemical larvicides, some authors have raised the possibility that larvivorous fish may harm indigenous species, including frogs and other fish species.

### Implications for research

This Cochrane Review provides limited research evidence that larvivorous fish can decrease immature mosquito populations in defined water bodies. This is hardly surprising as we know fish eat larvae, and in itself insufficient evidence to support investing in the intervention as a policy without further reliable research. What is unclear is whether this question is worth pursuing. Fish stocking is always going to be expensive, and the effects almost inevitably

will be marginal given the large numbers of water bodies usually present in areas where malaria-transmitting *Anopheles* lay eggs.

If researchers judge that this is a potentially effective intervention, then well-designed experimental studies to examine the effects on malaria in humans or, at the very least, on the EIR or the density of adult vector mosquitoes are required. It is important to note that researchers should carefully consider the design of the studies and should randomly allocate interventions to sites to minimize the risk of bias. In addition, researchers should undertake power calculations to decide the size of the study.

These studies should consider in the design any factors that could influence or bias the results (study design, baseline values, number of sites, pupae numbers reported, distance between sites, other larvivorous species present, vegetation cleared). Several effect modifiers had dramatic effects on immature forms, both within and between studies. This includes the ecological zones and settings, fish species, stocking density, and *Anopheles* species.

This research needs to be undertaken in a variety of ecological zones and settings, including household water sources, ponds, water canals, riverbed pools below dams, and rice fields, and should take into account the seasonality of malaria transmission in these study areas. Notably, testing the impact of fish in the absence of either LLINs or IRS would be unethical and against WHO-recommended policy. Therefore, the fish intervention would need tested in combination with the interventions of LLINs or IRS.

Research is required before larvivorous fish are used in malaria control, to be used either alone or in combination with other vector control methods. Furthermore, research studies should assess the environmental impact of larvivorous fish, particularly non-native introduced species, on the habitats into which they are released.

Apart from efficacy, questions remain regarding whether it is practical to deliver this method with the requisite quality and completeness of coverage on a larger scale than in experimental settings, whether it is cost-effective, whether it should be delivered as a stand-alone intervention or as an addition to IRS or LLINs, and whether this can be sustained for years.

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The findings and conclusions in this report have not been formally disseminated by the Centers for Disease Control and Prevention (CDC) and should not be construed as representing any agency determination or policy.

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* *Indicates the major publication for the study*

# CHARACTERISTICS OF STUDIES

## Characteristics of included studies *[ordered by study ID]*

### Fletcher 1992

| | |
|---|---|
| Methods | Study design: quasi-RCT<br>Study location: Assab Sekir and Negado Sefer, Assab, Ethiopia<br>Study dates: February 1987 to January 1988<br>Transmission intensity: endemic<br>Malaria parasite species: not specified<br>Primary vectors: *An. culicifacies adanensis*<br>Larval sites: domestic water containers<br>Baseline data: February 1987 |
| Participants | NA |
| Interventions | Fish species: *Aphanius dispar*<br>Indigenous fish species used: yes<br>Fish source: Gibdo River, 26 km from Assab<br>Populated sites: domestic water containers and wells; 68 stocked (32 barrels, 11 cisterns, 24 wells, 1 washbasin), 60 unstocked (33 barrels, 10 cisterns, 16 wells, 1 washbasin)<br>Restocked: yes, as necessary during surveys that were performed either monthly or every two weeks<br>Co-interventions: none |
| Outcomes | Percentage of larval sites positive for anopheline larvae<br>Method: standard dipping procedure; 5 dips/barrel, 12 dips/cistern, 8 dips/washbasin, 3 dips buckets/well during surveys that were performed either monthly or every two weeks |
| Source of funding | UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases; National Organisation for the Control of Malaria and Other Vectorborne Diseases, Ministry of Health, Ethiopia |
| Notes | No environmental data collected<br>Acceptability of fish to householders assessed by questionnaire |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Study design | High risk | Quasi-RCT: "In every other house or mosque, fish were stocked in all wells and water storage containers." |
| Site selection | Unclear risk | "A total of 54 households were selected by systematic sampling. All six mosques were also included in the study. Seven households were excluded because they had only jerrycans and buckets for water storage. |

**Fletcher 1992** (*Continued*)

| | | |
|---|---|---|
| | | They were replaced by seven other households selected by lottery system." |
| Site allocation | High risk | "In every other house or mosque, fish were stocked in all wells and water storage containers." |
| Blinding of outcome assessment (detection bias)<br>All outcomes | High risk | "During monthly or biweekly larval surveys the fish were counted and restocking was carried out as necessary to maintain the original number of fish." |
| Baseline values | Low risk | In both control and Intervention groups at prestocking (February 1987), the proportion of sites with *Anopheles* larvae was 0%. |
| Number of sites | Low risk | Number of sites adequate as > 20 sites per group. |

**Haq 2013**

| | |
|---|---|
| Methods | Study design: controlled time series<br>Study location: 2 villages, Pithai (intervention) and Anara (control), in Kheda district, Gujarat, India<br>Study dates: December 2010 to November 2011<br>Transmission intensity: endemic<br>Malaria parasite species: not specified<br>Primary vectors: *An. stephensi*<br>Larval sites: domestic water containers<br>Baseline data: July 2010. More than 100 houses in each village were checked |
| Participants | NA |
| Interventions | Fish species: *Aphanius dispar* (Rüppell)<br>Indigenous fish species used: yes<br>Fish source: collected from a natural habitat in a salt factory in the town of Cambay (Khambhat), Gujarat<br>Populated sites: 295 water storage containers, such as cement tanks including underground tanks (127), kothi (big mud pots), and barrels (167), in Pithai village. 30 containers in Pithai (intervention) and 25 in Anara (control) village monitored. Only cement tanks were included in longitudinal monitoring because of declining fish populations in other containers due to frequent replenishment<br>Restocked: no. Fish were released once during the 1-year study period, with 10 to 25 fish/tank or per container, depending on container size<br>Co-interventions: "routine intervention" |
| Outcomes | Density of immature *An. stephensi* stages (larvae instars I and II; III, IV and pupae) at weekly intervals for 4 weeks, then every 2 weeks. Only total % reduction in III/IV instar |

**Haq 2013** (*Continued*)

| | |
|---|---|
| | and pupae shown<br>Method: standard larval dipper method using the mean of 3 dips. Reduction in III and IV instar larvae and pupae was calculated as per the formula: % reduction = 100 - [(C1 × T2)/C2 × T1)] × 100 where: C1 = pre-release larval density in control tanks; C2 = post-release larval density in control tanks; T1 = pre-release larval density in fish tanks; and T2 = post-release larval density in fish tanks |
| Source of funding | Sardar Sarowar Narmadad Nigam Limited (SSNL), Gujarat |
| Notes | Correspondence with study author: "The same person/team counting the larval density were counting the fish density in tanks. The arbitrary presence of fishes was recorded in each tank and with the help of torch in under ground tanks". The study author was unable to provide raw data on number of fish or immature *Anopheles*. |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Study design | High risk | Controlled time series study |
| Site selection | Low risk | The trial authors selected 2 villages, Anara and Pithai, from 15 villages surveyed in Kheda district due to "the similar conditions in respect of type of domestic tanks, water supply and water storage practices." "Randomly, one of the Village Pithai was selected for introduction of *Aphanius* fish in all the tanks and water containers." |
| Site allocation | Unclear risk | The study introduced fish to 295 water storage containers, such as cement tanks including underground tanks (127), kothi (big mud pots), and barrels (167), in Pithai village. "The survival of the fish and mosquito larval was monitored in 30 containers in the experimental village and 25 in the control village." However, it is unclear how the trial selected which containers to monitor |
| Blinding of outcome assessment (detection bias)<br>All outcomes | High risk | The assessors were not blinded to treatment. "The survival of the fish and mosquito breeding was monitored...presence of fish was monitored with the help on a bright light torch." The study author stated via email that: "The same person/team counting the larval density were counting the fish density in tanks. The arbitrary presence of fishes was recorded in |

**Haq 2013** (*Continued*)

| | | |
|---|---|---|
| | | each tank and with the help of torch in under ground tanks. It was observed that 2-3 fishes were able to control the larval breeding may be because of the absence of alternate food in the domestic tanks filled with tap water." |
| Baseline values | Unclear risk | Baseline values for houses positive for mosquito larvae were comparable, but a higher number of containers were positive for mosquito in Anara (control) than in Pithai (Intervention) during baseline monitoring in July 2010 (container index 83.2 (Anara) versus 47.84 (Pithai)). It is unclear how comparable baseline values were before introduction of fish in the 2 villages in November/December 2010. We were unable to obtain further data from the corresponding study author due to "transfer from Nadiad to New Delhi HQ in 2012." |
| Number of sites | Low risk | Adequate numbers of sites in control and Intervention groups |

**Howard 2007**

| | |
|---|---|
| Methods | Study design: controlled interrupted time series<br>Study location: Kisii Central District, Western Kenya<br>Study dates: October 2003 to October 2004<br>Transmission intensity: endemic but highly seasonal<br>Malaria parasite species: not specified<br>Primary vectors: *An. gambiae s. l.*,*An. funestus* Giles<br>Larval sites: abandoned fishponds<br>Baseline data: October 2003 to January 2004 |
| Participants | NA |
| Interventions | Fish species: *Oreochromis niloticus* L.<br>Indigenous fish species used: yes<br>Fish source: local FD hatchery in Kisii town<br>Populated sites: 3 abandoned fishponds, Pond A (104 $m^2$), Pond C (128 $m^2$), and Pond D (72 $m^2$); 150 m distance from each other<br>Restocked: no<br>Co-interventions: none |
| Outcomes | Number of immature *Anopheles* per pond<br>Density of immature *Anopheles* per pond<br>Method: 5 larval dips (2.5 L total volume) randomly from edges of each pond, at least 1 dip/side, 5 to 7 days/week |

**Howard 2007** (*Continued*)

| | | |
|---|---|---|
| Source of funding | Government of Finland and BioVision | |
| Notes | Climatic data for study period obtained from Kenya Agricultural Research Institute<br>Study started with Pond B included, but as it was destroyed during the study period, the authors were unable to collect data for it for the requisite time period | |
| ***Risk of bias*** | | |
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Study design | High risk | Controlled interrupted time series study. |
| Site selection | Low risk | "The site has three abandoned fishponds within 150 m of each other." Author communication: "We started with a Pond B but it got destroyed during the study period so we were unable to collect data for it for the requisite time." |
| Site allocation | Unclear risk | Unclear how treatment for each site was chosen. |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether assessors were blinded to treatment. |
| Baseline values | Low risk | Numbers of *An. gambiae* s. l. and *An. funestus* immatures comparable in Ponds A, C, and D. |
| Number of sites | High risk | Probably inadequate as < 5 sites per group; control = 1 site, intervention = 2 sites |

**Imbahale 2011a**

| | |
|---|---|
| Methods | Study design: controlled time series<br>Study location: Nyalenda, Kisumu County, Kenya<br>Study dates: February 2008 to May 2008<br>Transmission intensity: not stated<br>Malaria parasite species: not specified<br>Primary vectors: *An. gambiae* Giles<br>Larval sites: man-made habitats (ponds or water canals)<br>Baseline data: not recorded |
| Participants | NA |

**Imbahale 2011a** (*Continued*)

| | |
|---|---|
| Interventions | Fish species: *G. affinis*<br>Indigenous fish species used: no<br>Fish source: colony at Kenya Medical Research Institute (KEMRI) established from a wild-caught population provided by Kenya Marine and Fisheries Research Institute (KEMFRI)<br>Populated sites: man-made habitats; 30 pools (mean 1 m × 1 m × 1 m deep) or water canals (15 m × 1 m × 0.3 m deep). Pond sites and water canal sites were constructed by people for the purposes of this experiment, so can be defined as "semi-field" studies<br>Restocked: no (treatment arm: ponds fish once), every 2 weeks (treatment arms: pond fish only or water canal fish only)<br>Co-interventions: *Bacillus thuringiensis* var. *israelensis* |
| Outcomes | Density of early instars (L1 and L2) or late instars (L3 and L4) of anopheline mosquitoes<br>Method: standard larval dipping procedure using 350 mL mosquito dipper (Bioquip, Gardena, CA, USA), maximum of 10 dips/habitat, estimated weekly |
| Source of funding | The Dioraphte Foundation, The Netherlands |
| Notes | |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Study design | High risk | Controlled time series study. |
| Site selection | Low risk | "Thirty man-made habitats (1 m × 1 m × 1 m) were created as mosquito larval habitats. " |
| Site allocation | Unclear risk | Unclear how treatment for each site was chosen for ponds. In water canals: "Six treatments were randomly administered in canal habitats." |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether assessors were blinded to treatment. |
| Baseline values | Unclear risk | Not reported. |
| Number of sites | High risk | Number of sites may be inadequate: 5 sites per group. |

**Kim 2002**

| | |
|---|---|
| Methods | Study design: controlled interrupted time series<br>Study location: Banwol, Suwon City, Gyeonggi Province, Korea<br>Study dates: June to October 1989<br>Transmission intensity: not specified<br>Malaria parasite species: not specified<br>Primary vectors: *An. sinensis*<br>Larval sites: rice fields<br>Baseline data: none |
| Participants | NA |
| Interventions | Fish species: *T. m. niloticus* (herbivorous) with either *A. latipes* or *Aphyocypris chinensis*<br>Indigenous fish species used: yes, except for *T. m. niloticus*<br>Fish source: *A. latipes*: not stated; *A. chinensis*: holding ponds at Ansan rice fields, 2.5 km north; *T. m. niloticus*: fish farm at Gwagiu, Gyeonggi<br>Populated sites: 6 rice fields (3 control sites, 3 intervention sites 500 m², 300 m², or 600 m² in size)<br>Restocked: no<br>Co-interventions: none |
| Outcomes | Mean number and percentage of reduction *An. sinensis*<br>Method: larval dips using 500 mL dipper, 2 to 4 replicates per rice field |
| Source of funding | Not stated |
| Notes | |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Study design | High risk | Controlled interrupted time series study. |
| Site selection | Unclear risk | "A confined field plot of ca. 20,000 m² rice field located in Banwol near Suwon City, Gyeonggi Province...three of the six paddies were taken." |
| Site allocation | Unclear risk | Unclear how treatment for each site was chosen for ponds. |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether assessors were blinded to treatment. |
| Baseline values | Low risk | Mean number of *An. sinensis* larvae comparable at Intervention and control sites. |
| Number of sites | High risk | Probably inadequate number of sites. |

**Kusumawathie 2008a**

| | |
|---|---|
| Methods | Study design: controlled before-and-after study<br>Study location: Kotmale oya, below Kotmale dam, Sri Lanka<br>Study dates: May to August 2000<br>Transmission intensity: epidemic<br>Malaria parasite species: not specified<br>Primary vectors: *An. culicifacies adanensis* (national importance), *An. annularis, An. subpictus, An. tessellatus* (local importance)<br>Larval sites: pools formed in riverbed between dam and power plant<br>Baseline data: 1 day before stocking |
| Participants | NA |
| Interventions | Fish species: *P. reticulata*<br>Indigenous fish species used: no<br>Fish source: riverbed pools below the Kotmale dam and then reared in stock tanks at Regional Office Anti-Malaria Campaign, Kandy<br>Populated sites: 60 riverbed pools, 0.25 to 1.0 m² surface area and < 1 m depth (29 intervention, 31 control, randomly selected)<br>Restocked: no<br>Co-interventions: none |
| Outcomes | Number (percentage) of pools positive for anopheline larvae<br>Mean number of larvae per pool<br>Mean number of larvae per 100 dips<br>Method: larval dipping using 100 mL dipper, 6 dips per m². Authors collected anopheline immatures but reported larval numbers only |
| Source of funding | National Research Council, Sri Lanka (NRC Grant No. 99/09) |
| Notes | Fish number monitored<br>*An. culicifacies* not identified at any sites |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Study design | High risk | Controlled before-and-after study. |
| Site selection | Unclear risk | "Sixty isolated riverbed pools...were selected and labeled." |
| Site allocation | Unclear risk | "*P. reticulata* was stocked in 29 randomly selected pools". Method of randomization not described |
| Blinding of outcome assessment (detection bias)<br>All outcomes | High risk | "Visual counts of *P. reticulata* were made in each pool, monthly. Visual counts were possible, as the pools were small (not exceeding 1 m² surface area), shallow (< 1 m |

**Kusumawathie 2008a** (*Continued*)

| | | |
|---|---|---|
| | | depth) and contained clean water." |
| Baseline values | Low risk | Comparable between control and intervention sites. |
| Number of sites | Low risk | Adequate numbers of sites in control (31 site) and intervention groups (29 sites) |

**Kusumawathie 2008b**

| | |
|---|---|
| Methods | Study design: controlled before-and-after study<br>Study location: riverbeds below Laxapana, Kotmale 1, Kotmale 2, Nilambe, Rantembe, and Victoria dams, Sri Lanka<br>Study dates: September 2000 to August 2002<br>Transmission intensity: epidemic<br>Malaria parasite species: not specified<br>Primary vectors: *An. culicifacies adanensis* (national importance), *An. annularis, An. subpictus*, and *An. tessellatus* (local importance)<br>Larval sites: pools formed in riverbed between dam and power plant<br>Baseline data: September 2000 to August 2001 |
| Participants | NA |
| Interventions | Fish species: *P. reticulata*<br>Indigenous fish species used: no<br>Fish source: not stated<br>Populated sites: pools of 6 riverbeds below dams (2 controls, 2 fish intervention)<br>Restocked: yes, pools that had no fish were restocked at the same rate during fortnightly larval surveys<br>Co-intervention: temephos treatment of all pools in 2 riverbeds |
| Outcomes | Mean percentage of pools positive for anopheline larvae<br>Mean number of anopheline larvae per 100 pools<br>Mean number of anopheline larvae per 100 dips<br>Total number of anopheline larvae<br>Methods: larval dips, 6 dips per $m^2$ surface area of water |
| Source of funding | National Research Council of Sri Lanka (Grant No. 99/09) |
| Notes | Cost analysis estimation and simulations performed |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Study design | High risk | Controlled before-and-after study. |

**Kusumawathie 2008b** (*Continued*)

| | | |
|---|---|---|
| Site selection | Low risk | "Six study sites, namely Laxapana, Kotmale 1, Kotmale 2, Nilambe, Rantembe and Victoria...were selected based on the occurrence of malaria outbreaks since 1985...all the pools in the riverbeds were stocked." |
| Site allocation | Unclear risk | Unclear how treatment for each site was chosen for ponds. |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether assessors were blinded to treatment. "Subsequently the pools that had no fish were restocked at the same rate." |
| Baseline values | High risk | Baseline values higher in intervention group than in control group |
| Number of sites | High risk | Probably inadequate: number of pools not specified. |

**Mahmoud 1985**

| | |
|---|---|
| Methods | Study design: controlled time series<br>Study location: Gezira irrigated area, Sudan<br>Study dates: January to December, but the years were not specified<br>Transmission intensity: not specified<br>Malaria parasite species: not specified<br>Primary vectors: *An. arabiensis*<br>Larval sites: small temporary pools<br>Baseline data: none |
| Participants | NA |
| Interventions | Fish species: *G. holbrooki* (note: this study refers to *G. affinis holbrooki*, as these fish were then considered a subspecies of *G. affinis*. This subspecies is now recognized as a full species)<br>Indigenous fish species used: no<br>Fish source: rearing ponds at Wad Medani, 20 to 25 km from trial sites<br>Populated sites: 20 irrigation canals, 1 m in depth, 2 m in width, and 4 to 10 km in length; 5 control canals<br>Restocked: yes<br>Co-intervention: none |
| Outcomes | Mean larval density of *An. arabiensis*/100 dips, according to instar stage<br>Methods: larval dipping at 2 sites per km in each canal, 10 dips per site |
| Source of funding | Malaria Control Project, Ministry of Health, Sudan |

**Mahmoud 1985** (*Continued*)

| | |
|---|---|
| Notes | Flow of water from large branch canals was controlled by gates opened at certain times; this system deprived the *Gambusia* of free movement into the smaller canals, which usually are richer in mosquito larvae than the larger ones, where the fish had originally been stocked |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Study design | High risk | Controlled time series study. |
| Site selection | Unclear risk | "Medium size irrigation canals of about 1 m depth, 2 m width, and 4-10 km length, officially classified as minor canals, were selected as sites for the trials. Twenty such canals were seeded with *Gambusia*...while five others were used as control." |
| Site allocation | Unclear risk | Unclear how treatment for each site was chosen for ponds. |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether assessors were blinded to treatment. |
| Baseline values | Unclear risk | Not reported. Fish release in October and measurements not taken until following January |
| Number of sites | High risk | May be inadequate, as only 5 sites in the control group. |

**Menon 1978**

| | |
|---|---|
| Methods | Study design: controlled interrupted time series study<br>Study location: Pondicherry Town, India<br>Study dates: January to May 1977<br>Transmission intensity: not specified<br>Malaria parasite species: not specified<br>Primary vectors: *An. stephensi*<br>Larval sites: wells, water tanks<br>Baseline data: January 1977 |
| Participants | NA |
| Interventions | Fish species: *G. affinis* or *A. blockii*<br>Indigenous fish species used: *G. affinis*: not indigenous, *A. blockii*: indigenous<br>Fish source: *G. affinis*: mass cultured at Vector Control Research Centre (VCRC); *A.* |

**Menon 1978** (*Continued*)

| | |
|---|---|
| | *blockii*: collected from ponds and stored at VCRC<br>Populated sites: 3402 to 3438 sites stocked; 317 sites unstocked<br>Restocked: yes; if no fish were present at sites at 1, 2, or 3 months after beginning of the trial, they were restocked with *G. affinis* or *A. blockii*<br>Co-intervention: none |
| Outcomes | Percentage of sites positive for anopheline larvae<br>Methods: bucket samples taken monthly |
| Source of funding | Not specified |
| Notes | Number of wells where fish survived monitored<br>Chemical analysis performed of water from wells where fish died (20) or survived (20) |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Study design | High risk | Controlled interrupted time series study. |
| Site selection | Low risk | "Every house with a well was marked in the experimental and comparison area." |
| Site allocation | Unclear risk | Unclear how treatment for each site was chosen for ponds. |
| Blinding of outcome assessment (detection bias)<br>All outcomes | High risk | "Wells were marked according to whether the fish was present or absent...it was possible to visually observe movement of *Gambusia* on the surface." |
| Baseline values | High risk | Not comparable between control and intervention sites. |
| Number of sites | Low risk | Adequate numbers of sites in control and intervention groups |

**Nalim 1988**

| | |
|---|---|
| Methods | Study design: controlled time series study<br>Study location: Central Java<br>Study dates: 1979 to 1984<br>Transmission intensity: endemic<br>Malaria parasite species: not specified<br>Primary vectors: not stated<br>Larval sites: rice fields<br>Baseline data: not recorded |
| Participants | NA |
| Interventions | Fish species: *C. carpio* and *P. reticulata*<br>Indigenous fish species used: *C. carpio*: indigenous, *P. reticulata*: not indigenous<br>Fish source: mass breeding of *C. carpio* in 9 ponds of 6 $m^2$ × 4 $m^2$ tended by fishery official in co-operation with village officials. Mass breeding of *P. reticulata* in 2 ponds of 4 $m^2$ × 2 $m^2$ tended by local fishery official.<br>Populated sites: number and size of control and intervention sites was not specified. Total size of area was 24.8 ha of wetland (rice fields), cultivated by 112 farmers<br>Restocked: fish were restocked every new rice planting season<br>Co-intervention: control area sprayed with fenitrothion at end of 1982 |
| Outcomes | Mean number newly emerged adult mosquitoes/$m^2$/day collected in traps (trap area 0.25 $m^2$) per year |
| Source of funding | TDR Grant UNDP/World Bank/WHO |
| Notes | |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Study design | High risk | Controlled time series study. |
| Site selection | Unclear risk | Number of fields not specified. "96.4% of the total 24.8 ha were included." |
| Site allocation | Unclear risk | Numbers of control and intervention sites not specified. Size of control area not specified |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether assessors were blinded to treatment. |
| Baseline values | Unclear risk | Not reported. |
| Number of sites | High risk | Probably inadequate, as number of sites not specified. |

**RTDC 2008**

| | |
|---|---|
| Methods | Study design: controlled time series<br>Study location: Vakhsh (Kirov 2 district) and Bokhtarskiy (Sadov 3 district) regions in Tajikistan<br>Study dates: 15 July to 21 August 2007<br>Transmission intensity: in 2007 there were no malaria cases in Saidov, and 5 cases in Kirov; but the study authors did not provide population denominator details<br>Malaria parasite species: not stated<br>Primary vectors: *Anopheles superpictus*, *Anopheles pulcherrimus*,*Anopheles hyrcanus*<br>Larval sites: rice fields<br>Baseline data: no baseline data |
| Participants | NA |
| Interventions | Fish species: *G. affinis*<br>Indigenous fish species used: no<br>Fish source: harvested from reservoirs noted to have *Gambusia*<br>Populated sites: rice field plots<br>Restocked: implied but not explicitly stated<br>Co-interventions: not described |
| Outcomes | Density of immature *Anopheles* mosquitoes by instar (data were not provided by species)<br>.<br>Method: authors used a standard net of 20 cm diameter. The net was immersed in water and held to 0.5 m in 1 direction, then taken in the opposite direction. The net contents were rinsed and the number of fish, and mosquito larvae and pupae counted. Five such samples gave the number of fish and the immature mosquitoes/m² |
| Source of funding | Not stated |
| Notes | No environmental data reported |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Study design | High risk | Controlled time series. |
| Site selection | High risk | Intervention and control areas each included 1 district with malaria cases and 1 district without malaria cases (no indication how sites for intervention and control areas were allocated) |
| Site allocation | Unclear risk | The study authors did not state how treatment was allocated to study sites |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Not possible to blind (presence of fish should be obvious); unclear if those who stocked the fish also sampled for larvae |

**RTDC 2008** (*Continued*)

| | | |
|---|---|---|
| Baseline values | Unclear risk | Study authors did not provide values for the interventions sites prior to introduction of fish |
| Number of sites | High risk | There were 2 sites in the intervention group and 2 in the control group |

**Sabatinelli 1991**

| | |
|---|---|
| Methods | Study design: controlled interrupted time series study<br>Study location: Grande Comore Island, Federal Islamic Republic of Comoros<br>Study dates: November 1987 to November 1988<br>Transmission intensity: endemic<br>Malaria parasite species: not specified<br>Primary vectors: *An. gambiae*<br>Larval sites: domestic water containers<br>Baseline data: November 1987 |
| Participants | NA |
| Interventions | Fish species: *P. reticulata*<br>Indigenous fish species used: not indigenous<br>Fish source: imported from Mayotte Island<br>Populated sites: domestic water containers; 20 unstocked (ablution basins) for duration of trial; 59 ablution basins and 61 tanks stocked in November 1987. Stocking of basins and tanks extended, and by April 1988, all basins and tanks were treated. Total numbers of basins and tanks stocked not specified<br>Restocked: not clearly indicated<br>Co-interventions: temephos (concentration: 2 mL/m$^3$) in tanks only, last treatment March 1988 |
| Outcomes | Percentage of containers positive for anopheline larvae<br>Method: surface and bottom of containers were examined for *An. gambiae* larvae (containers $\geq$ 15 cm in diameter), which were recorded monthly |
| Source of funding | Research was undertaken with the framework of project OMS-PNUD COM/MAL/001 |
| Notes | No environmental data collected |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Study design | High risk | Controlled interrupted time series study. |
| Site selection | Unclear risk | Unclear how sites were selected. |

**Sabatinelli 1991** (*Continued*)

| | | |
|---|---|---|
| Site allocation | Unclear risk | Unclear how intervention treatment was selected. Control sites were in village of Bandamadji, 3 km from intervention site |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether assessors were blinded to treatment. |
| Baseline values | Low risk | Percentage of sites positive for *An. gambiae* larvae comparable in control and Intervention groups. |
| Number of sites | Low risk | Adequate numbers of sites in control and Intervention groups |

**Sitaraman 1976**

| | |
|---|---|
| Methods | Study design: controlled interrupted time series study<br>Study location: Great Hyderabad City, India<br>Study dates: not stated<br>Transmission intensity: endemic<br>Malaria parasite species: not specified<br>Primary vectors: *An. stephensi*<br>Larval sites: domestic water containers<br>Baseline data: day 0, before release of fish |
| Participants | NA |
| Interventions | Fish species: *P. reticulata*<br>Indigenous fish species used: not indigenous<br>Fish source: not stated<br>Populated sites: 5 control and 12 intervention (50 guppies/well); 4 control and 10 intervention (100 guppies/well)<br>Restocked: no<br>Co-interventions: temephos (concentration: 2 mL/m$^3$) |
| Outcomes | Density of immature *An. stephensi* stages (larvae instars I and II, III and IV, pupae)<br>Method: 5 dips per well using a 30 cm diameter net |
| Source of funding | Not stated |
| Notes | |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|

**Sitaraman 1976** (*Continued*)

| | | |
|---|---|---|
| Study design | High risk | Controlled interrupted time series study. |
| Site selection | Unclear risk | Unclear how these particular sites were selected. |
| Site allocation | Unclear risk | Unclear how treatment was allocated. |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether assessors were blinded to treatment. |
| Baseline values | High risk | Mean values not comparable between control and intervention groups |
| Number of sites | High risk | Numbers of sites may be inadequate as 4 control sites were used |

**Yu 1989**

| | |
|---|---|
| Methods | Study design: controlled interrupted time series study<br>Study location: Korea<br>Study dates: June to September 1988<br>Transmission intensity: not specified<br>Malaria parasite species: not specified<br>Primary vectors: *An. sinensis*<br>Larval sites: rice fields<br>Baseline data: June to August 1988 |
| Participants | NA |
| Interventions | Fish species: *A. latipes* and *T. m. niloticus*<br>Indigenous fish species used: *A. latipes*: indigenous; *T. m. niloticus*: not indigenous<br>Fish source: *A. latipes* originated from holding ponds at Ansan rice fields (2.5 km away) , *T. m. niloticus* sourced from fish farm in Jin-Dong of Masan City, South Kyungsang Province<br>Populated sites: rice fields (2 control sites, 2 intervention sites with *A. latipes* and *T. m. niloticus,* 2 intervention sites with *A. latipes* only, followed by *Bacillus thuringiensis* treatment after 3 weeks)<br>Restocked: no<br>Co-interventions: see above |
| Outcomes | Density of *An. sinensis* larvae determined weekly<br>Method: larval dipping performed using a 500 mL dipper, 2 to 4 replicates per rice field usually consisting of 2 dips pooled |
| Source of funding | WHO Medical Research Fund of the Western Pacific Region, Manila |
| Notes | Environmental data (temperature and rainfall) recorded |

**Yu 1989** (*Continued*)

| *Risk of bias* | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Study design | High risk | Controlled interrupted time series study. |
| Site selection | Low risk | "A confined field plot of ca 1,000 m²...the rice paddy was composed of 6 similar sized (10 × 15 × 0.3 m) plots." |
| Site allocation | Unclear risk | "2 random selection of paddies was made for each group." Method of random selection not specified |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Unclear whether assessors were blinded to treatment. |
| Baseline values | Low risk | Comparable between control and intervention sites. |
| Number of sites | High risk | Probably inadequate number of sites. |

**Zvantsov 2008**

| | |
|---|---|
| Methods | Study design: controlled time series<br>Study location: Farkhor district (Kizilpakhtachi village) and Shaartuz district (Birlyash village), Tajikistan<br>Study dates: Kizilpakhtachi village 25 June to 29 August 2008; Birlyash village: 25 June to 26 August 2008<br>Transmission intensity: not mentioned<br>Malaria parasite species: not mentioned<br>Primary vectors: *An. superpictus*<br>Larval sites: rice fields<br>Baseline data: reported values measured immediately before introduction of fish |
| Participants | NA |
| Interventions | Fish species: *G. affinis*,*P. reticulata*<br>Indigenous fish species used: no.<br>Fish source: *G. affinis,* not mentioned; *P. reticulata* bred in basic laboratory at the Republican Centers for Combating Tropical Diseases (RCCTD)<br>Populated sites: in each paddy field, 9 rice field checks (3 m x 3 m) were used: 3 filled with *P. reticulata*, 3 with *G.* affinis, and 3 served as controls. Checks with the different species of fish were arranged in a chequer board pattern<br>(A rice check is a square or rectangular area of a paddy field created by low, narrow banks of earth (dykes) that serve to divide the paddy field into manageable areas and to control the flow of water.) |

| | |
|---|---|
| | Restocked: yes, but unclear whether for *P. reticulata* alone due to poor survival or both *P. reticulata* and *G. affinis*. Graphs indicated that fish were introduced twice, but text stated "Because of the problem of using guppies as larviphages, related to their much worse survival rate in the native conditions in Tajikistan than the survival rate of gambezi (which can safely be regarded as a representative of the local ichthyofauna), it was necessary to re-release guppies into the rice checks to reduce the number of larvae."<br>Co-interventions: not stated |
| Outcomes | Outcome: density of either younger or older *Anopheles* larvae/m²<br>Method of measurement: a 20 cm diameter net, or a photographic cuvette, was immersed in the water to half-way down the rim, swept for 1 m to 1 side, trawling the superficial layers, then turned sharply and swept the other way for 1 m to trawl the bottom layers. Net contents were rinsed into a cuvette and the numbers of fish and mosquito larvae and pupae counted. 5 such samples will give the number of fish and pre-imago mosquitoes per m² |
| Source of funding | No information provided |
| Notes | Article in Russian. Data were estimated from graphs<br>In intervention checks *G. affinis* multiplied successfully despite the presence of predators dragonfly larvae, water bugs, water beetles, marsh frogs. *P. reticulata* had lower survival in the field than *G. affinis*. |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Study design | High risk | Controlled time series. |
| Site selection | Unclear risk | Study authors did not state how they selected sites. |
| Site allocation | Low risk | Checks with *Gambusia* or *Poecilia* or control were arranged in a chequer board pattern. Study authors stated that allocation was random |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Presence of fish was obvious, but it was not clear whether observers of larval densities were blinded |
| Baseline values | Unclear risk | The study authors reported baseline values taken immediately before introduction of fish. Baseline values were comparable in the Birlyash village, but not in the Kizilpakhtachi village. Authors reported mean values only |

**Zvantsov 2008** (*Continued*)

| | | |
|---|---|---|
| Number of sites | High risk | Study authors used 2 sites, each comprised 3 checks for control, 3 for *G. affinis*, and 3 for *P. reticulata.* |

Abbreviations: NA: not applicable; RCT: randomized controlled trial; UNDP: United Nations Development Programme; WHO: World Health Organization.

## Characteristics of excluded studies *[ordered by study ID]*

| Study | Reason for exclusion |
|---|---|
| Alio 1985a | Transmission baseline data collected for < 1 year pre-intervention. For larval population data, *Anopheles* and *Culex* populations not monitored separately. |
| Alio 1985b | Not a fish trial. Review article. |
| Asimeng 1993 | Not a fish trial. |
| Austen 1919 | Not a fish trial. Review article. |
| Azevedo-Santos 2016 | A commentary on use of larvivorous fish to control *Aedes* mosquitoes. |
| Bang 1988 | Not a fish trial. Review article. |
| Bay 1967 | Not a fish trial. Review article. |
| Bedford 1938 | Medical report, not a fish trial. |
| Beltran 1973 | Not a fish trial. Review article. |
| Bolay 1989 | No primary or secondary outcomes. |
| Borel 1926 | No primary or secondary outcomes. |
| Brumpt 1928 | Not a controlled trial. |
| Caillouet 2008 | Not a fish trial. |
| Carlson 2004 | Not a fish trial. |
| Carnevale 1990 | Not a fish trial. Review article. |
| Chandra 2008 | Not a fish trial. Review article. |

(*Continued*)

| | |
|---|---|
| Chandra 2013 | Not a fish trial. Review article. |
| Chapman 1974 | Not a fish trial. Review article. |
| Coulon 1931 | Not a controlled trial. |
| Das 1991 | *Anopheles* and *Culex* populations not monitored separately. No primary outcomes. |
| de Buen 1930 | Unclear study design. Unclear whether control sites were true controls or areas of no *Gambusia* fish in the same water body. As this study was published in 1930, we were unable to contact the study author for further details |
| De Burca 1939 | Not a fish trial. Descriptive article. |
| Dev 2008 | Not a fish trial. Descriptive article. |
| Devi 2010 | No primary or secondary outcomes. |
| Dua 1991 | Multiple interventions, cannot determine effect of fish alone |
| Dua 1997 | Multiple interventions, cannot determine effect of fish alone |
| Fletcher 1993 | Laboratory-based study only. |
| Gammans 1926 | Not a fish trial. |
| Ghosh 2005 | Inappropriate study design. |
| Ghosh 2007 | Not a fish trial. Review article. |
| Ghrab 1999 | Laboratory-based study only. |
| Gupta 1989 | Not a fish trial. |
| Gupta 1992 | *Anopheles* and *Culex* populations not monitored separately. No primary outcomes. |
| Haas 1984 | Not a fish trial. |
| Hackett 1938 | Not a fish trial. Review article. |
| Hadjinicolaou 1973 | Inappropriate study design. |
| Holland 1933 | No primary or secondary outcomes. |
| Homski 1994 | Laboratory-based study only. |
| Howard 1920 | Inappropriate study design. |

(*Continued*)

| | |
|---|---|
| Hurlbert 1972 | No primary or secondary outcomes. |
| Imbahale 2011b | Not a fish trial. Review article. |
| Inci 1992 | Inappropriate study design. |
| Jayawardana 2001 | Inappropriate study design. |
| Julvez 1987 | Inappropriate study design. |
| Kaneko 2000 | Inappropriate study design. |
| Kligler 1930 | Not a fish trial. |
| Kondrashin 2017 | Study authors reported *Anopheles* and *Culex* immature mosquito numbers combined. |
| Kumar 1998 | Inappropriate study design. |
| Kusumawathie 2006 | Laboratory-based study only. |
| Lacey 1990 | Not a fish trial. Review article. |
| Legendre 1921 | Inappropriate study design. |
| Louis 1988 | Inappropriate study design. |
| Luh 1981 | Inappropriate study design. |
| Malhotra 1992 | Inappropriate study design. |
| Mandoul 1954 | Inappropriate study design. |
| Manimunda 2009 | Not a controlled trial. |
| Menon 1977 | Inappropriate study design. |
| Merle 1955 | Inappropriate study design. |
| Missiroli 1930 | Inappropriate study design. |
| Mohamed 2003 | Inappropriate study design. |
| Molloy 1924 | Inappropriate study design. |
| Morin 1936 | Inappropriate study design. |
| Nalim 1987 | No primary outcomes. Secondary outcomes in Nalim 1988. |

(*Continued*)

| | |
|---|---|
| Ossi 1984 | Inappropriate study design. |
| Panicker 1985 | Inappropriate study design. |
| Patra 2010 | *Anopheles* and *Culex* populations not monitored separately. No primary outcomes. |
| Pecori 1930 | Inappropriate study design. |
| Prasad 1993 | Inappropriate study design. *Anopheles* and *Culex* populations not monitored separately. |
| Pyke 2008 | Not a fish trial. Review article. |
| Raina 1945 | Inappropriate study design. |
| Rajnikant 1993 | Inappropriate study design. *Anopheles* and *Culex* populations not monitored separately. |
| Rao 1942 | Inappropriate study design. |
| Rimbaut 1935 | Inappropriate study design. |
| Robert 1998 | Inappropriate study design. |
| Rojas 2004 | Inappropriate study design. |
| Roule 1934 | Inappropriate study design. |
| Roy 1938 | Inappropriate study design. |
| Rupp 1996 | Inappropriate study design. |
| Russell 1942 | Inappropriate study design. |
| Sabatinelli 1988 | No primary outcomes. Secondary outcomes in Sabatinelli 1991. |
| Sella 1927 | Inappropriate study design. |
| Sella 1929 | Inappropriate study design. |
| Sergiev 1937 | Inappropriate study design. |
| Sharma 1986a | Inappropriate study design. |
| Sharma 1986b | Multiple interventions, cannot determine effect of fish alone |
| Sharma 1989a | Inappropriate study design. |

(*Continued*)

| | |
|---|---|
| Sharma 1989b | Multiple interventions, cannot determine effect of fish alone |
| Sharma 1991 | Multiple interventions, cannot determine effect of fish alone |
| Sharma 1997 | No primary outcomes. Secondary outcome follow-up only 3 weeks in duration |
| Singh 1989 | Multiple interventions, cannot determine effect of fish alone |
| Singh 2006 | Multiple interventions, cannot determine effect of fish alone |
| Sitaraman 1975 | Inappropriate study design. No control area. |
| Sunish 2015a | Not a controlled trial. |
| Sunish 2015b | Not a controlled trial. |
| Tabibzadeh 1970 | Not a fish trial. |
| Teklehaimanot 1993 | Not a fish trial. |
| Tisohlbr 1950 | Inappropriate study design. |
| Trausmiller 1932 | Inappropriate study design. |
| Ungureanu 1981 | Not a fish trial. A manual on how to evaluate fish. |
| Usenbaev 2006 | Inappropriate study design. |
| Van Dam 2007 | Inappropriate study design. Not in malaria-endemic area. |
| Velichkevich 1935 | Inappropriate study design. |
| Victor 1994 | Not a fish trial. |
| Vitlin 1987a | Inappropriate study design. |
| Vitlin 1987b | Inappropriate study design. |
| Walton 2007 | Not a fish trial. Review article. |
| Warbanski 2017 | Study authors reported number of immature mosquitoes, and not specifically anopheline mosquitoes |
| Wickramasinghe 1986 | Not a fish trial. Review article. |
| Wu 1991 | *Anopheles* and *Culex* populations not monitored separately. Inappropriate study design |
| Yadav 1993 | Inappropriate study design. Multiple interventions, cannot determine effect of fish alone |

(*Continued*)

| | |
|---|---|
| Yu 1982a | Inappropriate study design. |
| Yu 1982b | Secondary outcomes in Yu 1982a. |
| Yu 1982c | Secondary outcomes in Yu 1982a. |
| Yu 1986 | Inappropriate study design. Culex monitored only. |
| Zaman 1980 | Inappropriate study design. Laboratory-based experiment only |

## DATA AND ANALYSES

This review has no analyses.

## ADDITIONAL TABLES

**Table 1. 'Risk of bias' assessment**

| Risk of bias factor | Risk of bias | | |
|---|---|---|---|
| | High | Low | Unclear |
| 1. Study design | Non-RCT | RCT | Not clearly reported or not reported |
| 2. Site selection | Method of selection of sites within study area not described | Method of selection of sites within study area described | Not clearly reported or not reported |
| 3. Site allocation | Allocation of treatment not performed by random allocation | Allocation of treatment performed by random allocation | Not clearly reported or not reported |
| 4. Blinding of assessors | Not blinded | Blinded | Not clearly reported or not reported |
| 5. Baseline values comparable between sites | Not comparable | Comparable | Not clearly reported or not reported |
| 6. Number of sites | May be inadequate (5 to < 20 sites per group)<br>Probably inadequate (< 5 sites per group or number of sites unknown) | Adequate number of sites (≥ 20 sites per group) | Not clearly reported or not reported |

Abbreviations: RCT: randomized controlled trial.

**Table 2. Ecological sites classified by site type, with a description of number of sites and their size**

| Group | Site type | Study | Sites stocked | Unstocked | Site size | |
|---|---|---|---|---|---|---|
| | | | | | Surface area | Depth |
| Localized water bodies[1] | Wells | Menon 1978 | 3402 to 3438 | 317 | Not stated | Not stated |
| | | Sitaraman 1976 | 10 | 4 | 1.5 m² | 1.5 to 2.5 m |
| | Domestic water containers | Fletcher 1992[2] | 68 | 60 | Not stated | Not stated |
| | | Haq 2013[3] | 295 (30 monitored) | 25 monitored | Not stated | Not stated |

**Table 2. Ecological sites classified by site type, with a description of number of sites and their size** (*Continued*)

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Sabatinelli 1991[4] | 120[5] | 20 | Not stated | Not stated |
| | Fishponds and man-made pools | Howard 2007[6] | 2 | 1 | 72 m² to 128 m² | Not stated |
| | | Imbahale 2011a[7] | 25 | 5 | Mean 1 m² | 1 m |
| | Riverbed pools below dams | Kusumawathie 2008a | 29 | 31 | 0.25 to 1 m² | < 1 m |
| | | Kusumawathie 2008b | 2 areas. Number of sites unknown | 2 areas. Number of sites unknown | Not stated | Not stated |
| Rice field plots | | Kim 2002 | 3 | 1 | 300 m² to 600 m² | Not stated |
| | | Nalim 1988 | Not specified | Not specified | 23.9 ha in total | Not stated |
| | | RTDC 2008 | 2 | 2 | Not stated | Not stated |
| | | Yu 1989 | 4 | 2 | 45 m³ | 0.01 m |
| | | Zvantsov 2008 | 2 areas, with 6 checks[8] in 1 paddy field per area (3 checks treated with *Gambusia affinis*, 3 checks treated with *Poecilia reticulata*) | 2 areas. 3 checks in 1 paddy field per area | Each paddy field had 9 checks, and each check was 3 m × 3 m | Not stated |
| Water canals | | Imbahale 2011a | 25 | 5 | Mean 15 m² | 0.3 m |
| | | Mahmoud 1985 | 20 | 5 | 4 km to 10 km × 2 m wide | 1 m |

[1]Included wells, domestic water containers, fishponds and man-made pools, and riverbed pools below dams.
[2]Included barrels, cisterns, wells, and washbasins.
[3]Included cement tanks, including underground tanks, kothi (big mud pots), and barrels.
[4]Included ablution basins and tanks.
[5]Number of sites at follow-up in November 1987; Sabatinelli 1991 did not specify the number sampled at the April 1988 follow-up.
[6]Included fishponds only.
[7]Included man-made pools only.
[8]A rice check is a square or rectangular area of a paddy field created by low, narrow banks of earth (dykes) that serve to divide the paddy field into manageable areas and to control the flow of water.

**Table 3. Details of the fish intervention**

| Study | Fish species introduced | Stocking density | Type of site | Size of site | Size (maturity) of fish | Sex ratio male: female | Time of year fish introduced | Restocked |
|---|---|---|---|---|---|---|---|---|
| Fletcher 1992 | *Aphanius dispar* | 5 fish per barrel, 10 fish per cistern, 20 fish per well, 60 fish per washbasin; later, 10 fish per barrel and 40 fish per well | Domestic water containers | Not stated | Not stated | Not stated | February | Yes |
| Haq 2013 | *A. dispar* | 10 to 25 fish per tank or container, depending on the container size | Domestic water containers | Not stated | Not stated | Not stated | November to December | No |
| Howard 2007 | *Oreochromis niloticus* | 2 fish per m² pond surface area | Abandoned fishponds | 104 m² (pond A), 128 m² (pond C), 72 m² (pond D) | 1 to 2 months old | Not stated | January | No |
| Imbahale 2011a | *G. affinis* | Total number based on feeding rate of 4 mosquito fish per 60 mosquito larvae per day | Man-made pools or water canals | Pools (mean 1 m × 1 m × 1 m deep) or water canals (15 m × 1 m × 0.3 m deep) | 4 cm to 7 cm | Not stated | February | No (treatment arm: ponds fish once). Yes, every 2 weeks (treatment arms: pond fish only or water canal fish only) |
| Kim 2002 | (1) *A. latipes* with *T. m. niloticus*. (2) *Aphyocypris chinensis* + *T.* | (1) 1 pair *T. m. niloticus*/10 m² water surface + 0.8 *A. latipes*/m² water | Rice fields. | Rice fields (1) 500 m², (2) 300 m², or 600 m². | Not stated. | Not stated. | June. | No. |

**Table 3. Details of the fish intervention** (*Continued*)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *m. niloticus.* | surface. (2) 1 *A. chinensis*/m² + 2 *T. m. niloticus*/ 10 m². | | | | | | |
| Kusumawathi 2008a | *P. reticulata.* | 5 fish/m² surface area. | Riverbed pools below dams. | 0.25 to 1 m² surface area and < 1 m depth. | Not stated. | 2:3 | May. | No. |
| Kusumawathi 2008b | *P. reticulata* | 5 fish/m² surface area | Riverbed pools below dams | Not stated | Not stated | 2:3 | August | Yes |
| Mahmoud 1985 | *G. holbrooki* | Unclear. Authors stated a total of 8000 to 12,000 fish per canal depending on length and 1000 fish | Canals | 1 m depth, 2 m width, 4 to 10 km length | Not stated | Not stated | October | Yes |
| Menon 1978 | *G. affinis* and *A. blockii* | 20 fish per negative well, 50 fish per positive well | Wells | Not stated | Not stated | Not stated | January | Yes |
| Nalim 1988 | *P. reticulata* and *C. carpio* | 9 *C. carpio*/ 10 m² and 2 *P. reticulata*/ m² | Rice fields | 23. 9 ha in total, but size of individual ponds not specified | Not stated | Not stated | Not stated | Yes |
| RTDC 2008 | *G. affinis* | Not clearly stated; study authors reported from 2 to 3 fish/m² (1st timepoint) up to 15 to 18 fish/ m² (Vakhsh, | Rice fields | Not stated | Not stated | Not stated | Not stated | Not clearly indicated |

**Table 3. Details of the fish intervention** (*Continued*)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Kirov 2) or 18 to 20 fish/m² (Bokhtarskiy, Sadov 3 districts) | | | | | | |
| Sabatinelli 1991 | *P. reticulata* | 3 to 5 fish/m $^3$ | Domestic water containers | Size of domestic water containers (ablution basins and tanks) not clearly indicated | Not stated | Not stated | November | Not clearly indicated |
| Sitaraman 1976 | *P. reticulata* | Either 50 or 100 fish per well | Wells | 1.5 to 2.5 m depth, average square area 1.5 m² | Not stated | Not stated | Not stated | No |
| Yu 1989 | *A. latipes* and *T. m. niloticus* | 2 *A. latipes*/m² and 2 *T. m. niloticus*/10 m² or 2 *A. latipes*/m² only | Rice fields | Each plot was 10 × 15 × 0.3 m, depth 10 cm | Not stated | Not stated | June | No |
| Zvantsov 2008 | *G. affinis* or *P. reticulata* | 5 pregnant females/m² (total of 45 females per 3 m × 3 m check) | Rice fields | Each paddy field had 9 checks, and each check was 3 m × 3 m | Only stated as adult and pregnant | Not stated | June to August | Yes, but unclear whether *P. reticulata* alone or both *P. reticulata* and *G. affinis*. Graphs indicated that both species of fish could have been introduced twice, but text stated, "Because of the problem |

**Table 3. Details of the fish intervention** (*Continued*)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | of using guppies as larviphages, related to their much worse survival rate in the native conditions in Tajikistan than the survival rate of gambezi (which can safely be regarded as a representative of the local ichthyofauna), it was necessary to re-release guppies into the rice checks." |

**Table 4. Design quality**

| Study ID | Pupae numbers reported | Distance between sites | Other larvivorous species present | Vegetation cleared |
|---|---|---|---|---|
| Fletcher 1992 | Recorded but not reported | < 1 km | Not reported | Not reported |
| Haq 2013 | Only % reduction of L3 to L4 larvae and pupae combined reported | 13 km | Not reported | Not reported |
| Howard 2007 | Only larvae and pupae combined reported | < 1 km | Not reported | Three ponds cleared of vegetation on a weekly basis |
| Imbahale 2011a | Not reported | Not reported | Not reported | Not reported |
| Kim 2002 | Not reported | < 1 km | Not reported for control site. For treatment site, no other larvivorous fish | Herbivorous fish *Tilapia mossambicus niloticus* used at intervention but |

**Table 4. Design quality** (*Continued*)

| | | | | |
|---|---|---|---|---|
| | | | found | not control sites |
| Kusumawathie 2008a | Recorded but not reported | < 1 km | Not reported | Not reported |
| Kusumawathie 2008b | Not reported | Not reported | Not reported | Not reported |
| Mahmoud 1985 | Not reported | Not reported | Not reported | Not reported |
| Menon 1978 | Not reported | Not reported | Not reported | Not reported |
| Nalim 1988 | Not reported | Not reported | Not reported | Not reported |
| RTDC 2008 | Yes | Not reported | Not reported | Not reported |
| Sabatinelli 1991 | Not reported | 3 km | Not reported | Not reported |
| Sitaraman 1976 | Yes | Not reported | Not reported | Not reported |
| Yu 1989 | Not reported | < 1 km | Not reported | Herbivorous fish *T. m. niloticus* used in 1 study arm only |
| Zvantsov 2008 | Recorded but only larvae reported | Intervention and control in same paddy field in each site | Assessed, but not reported | Not clearly reported |

**Table 5. Summary of included studies**

| Site type | | Study | Intervention | Outcome | Result |
|---|---|---|---|---|---|
| Localized water bodies | Wells | Menon 1978 | Intervention: *Gambusia* or *Aplocheilus* fish to 3438 wells; 50 fish per well if anopheline larvae present; 20 fish per well if no larvae present<br>Control: 317 wells | Percentage of sites with *An. stephensi* larvae up to 4 months' follow-up | Study appeared to provide evidence of a larvicidal effect of fish in wells using relatively high fish stocking levels |
| | | Sitaraman 1976 | 100 *P. reticulata* per well<br>Intervention: 10 wells<br>Control: 4 wells<br>50 *P. reticulata* per | *A. stephensi* larval and pupal densities up to 28 days (100 fish per well) or 22 days (50 fish per well) | At high fish stocking levels, larvae were eliminated in the first 4 days in wells but reappeared at lower levels from |

**Table 5. Summary of included studies** (*Continued*)

| | | | | | |
|---|---|---|---|---|---|
| | | | well<br>Intervention: 12 wells<br>Control: 5 wells | | day 24 onwards<br>With lower fish stocking levels, there was a partial effect for 2 weeks only, with rebound |
| | Wells and domestic water containers | Fletcher 1992 | Intervention: *Aphanius dispar* (60 sites)<br>Control: 51 sites | Percentage of sites with *An. culicifacies adanensis* larvae up to 11 months' follow-up | Study provided evidence that fish introduction prevents an increase in the number of domestic water container sites with larvae compared with control up to 11 months' follow-up |
| | | Haq 2013 | Intervention: *A. dispar* (295 water containers, of which 30 were monitored)<br>Control: 25 containers | Percentage reduction in *An. stephensi* L3-L4 larvae and pupae up to 12 months' follow-up | Study appeared to provide evidence that fish introduction reduces the number of L3-L4 larvae and pupae in domestic water containers compared with control up to 12 months' follow-up |
| | | Sabatinelli 1991 | Intervention: *P. reticulata* fish (59 sites in November 1987, total number of sites not specified)<br>Control: 20 ablution basins | Percentage of containers positive for *An. gambiae* larvae for 11 months' follow-up | Study appeared to show that fish reduce the number of domestic wash basins with larvae when added to these sites for up to 11 months |
| | Fishponds and pools | Howard 2007 | Intervention: *Oreochromis niloticus* fish (2 ponds)<br>Control: 1 pond | Number of immature *An. gambiae* and *An. funestus* mosquitoes for 5 months' follow-up | Based on trends in the study authors' graph, data that we extracted from the graph, and the study authors' analysis, this study appeared to provide limited evidence of a possi- |

**Table 5. Summary of included studies** (*Continued*)

| | | | | | |
|---|---|---|---|---|---|
| | | | | | ble larvicidal effect of fish in ponds |
| | | Imbahale 2011a | See the water canals section below. | | |
| | Riverbed pools below dams | Kusumawathie 2008a | Intervention: *P. reticulata* (29 riverbed pools) Control: 31 pools | Percentage of pools with *Anopheles* larvae, mean number of *Anopheles* larvae per pool, and mean number of *Anopheles* larvae per 100 dips up to 120 days' follow-up | At follow-up, the intervention group had greater reductions than the control group for the outcomes of percentage of pools with *Anopheles* larvae, mean number of larvae per pool, and mean number of larvae per 100 dips |
| | | Kusumawathie 2008b | Intervention: *P. reticulata* to all riverbed pools in Laxapana and Kotmale (1 study site) Control: all riverbed pools in Kotmale 2 and Nilambe | Percentage of pools with *Anopheles* larvae, mean number of *Anopheles* larvae per pool, and mean number of *Anopheles* larvae per 100 dips up to 1 year follow-up | At follow-up, riverbed pools stocked with fish had larger reductions in terms of presence and density of larvae |
| Rice field plots | | Kim 2002 | Intervention: *Tilapia mossambicus* and *A. latipes* (treatment A, 1 rice field plot) or *A. chinensis* and *Tilapia mossambicus* (treatment B and treatment C, 1 rice field plot each) Control: 3 rice field plots of similar size | Number of *An. sinensis* larvae up to 13 weeks' (treatment A) or 7 weeks' (treatment B and C) follow-up | In the control group and with treatments B and C, the number of *An. sinensis* larvae was higher at 2 weeks' pre-intervention than at 6 weeks' pre-intervention. At 2 weeks' follow-up, the *An. sinensis* larval population in the control group was the same as at 2 weeks' pre-intervention, but decreased at 6 weeks' follow-up. Larvae were clearly reduced |

**Table 5. Summary of included studies** (*Continued*)

| | | | | |
|---|---|---|---|---|
| | | | | at the 2 sites where fish were introduced For treatment A, the number of *An. sinensis* larvae increased between one week' and five weeks' follow-up at both control and intervention sites. However, the number of larvae decreased by 13 weeks' follow-up at both control and intervention sites. This shows a mean difference in larvae density between control and intervention over the entire period of observation. However, these data were weaker, as no baseline density was noted in the intervention arm, and any difference from the control could be due to chance |
| | | Nalim 1988 | Intervention: 23.9 ha of rice fields with *P. reticulata* and *C. carpio* fish<br>Control: did not specify the size of the control area used<br>Total numbers of control and Intervention field plots not specified | Number of *An. aconitus*, *An. barbirostris*, and *An. annularis* newly emerged adult mosquitoes collected/m²/day (trap area = 0.25 m²) up to 6 years' follow-up | Effects were mixed, with some indication of an effect of fish on *An. aconitus* and *An. annularis*, but not on *An. barbirostris.* |
| | | RTDC 2008 | Intervention: 2 rice field plots treated with *G. affinis* fish<br>Control: 2 rice field plots | Number of *Anopheles* larvae and pupae up to 40 or 41 days' follow-up | Study appeared to provide evidence of a larvicidal effect of fish in rice field plots up to 40/41 days' follow-up |

**Table 5. Summary of included studies** (*Continued*)

| | | | | | |
|---|---|---|---|---|---|
| | | Yu 1989 | Intervention: 2 plots treated with 2 species of fish (*A. latipes* and *Tilapia mossambicus*), 2 plots treated with 1 species alone (*A. latipes*) Control: 2 plots | Number of *An. sinensis* larvae up to 4 weeks' (1 fish) or 7 weeks' (2 fish) follow-up | At 4 weeks, larvae had increased against baseline in both control and intervention plots, but the size of the increase was lower in the 2 plots treated with 1 species Follow-up at 4 weeks and 7 weeks showed considerably lower values in the 2 plots treated with 2 species than in the control |
| | | Zvantsov 2008 | 2 areas, 1 rice field per area Intervention: per rice field, 3 checks treated with *G. affinis*, and 3 treated with *P. reticulata* Control: 3 untreated checks per rice field (a rice check is a square or rectangular area of a paddy field created by low, narrow banks of earth (dykes) that serve to divide the paddy field into manageable areas and to control the flow of water) | Density of "younger" or "older" *Anopheles* larvae per m² up to 62 days' (Birlyash village) or 65 days' (Kizilpakhtachi village) follow-up | Based on data that we extracted from the study authors' graphs, this study appears to provide limited evidence of a possible larvicidal effect of *G. affinis* fish in the rice field plots of both areas studied. *P. reticulata* reduced the larval density to similar levels as *G. affinis* in 1 district, but the effect was less sustained compared to *G. affinis* in the Shaartuz district, Birlyash village. |
| Water canals | | Imbahale 2011a | Ponds Intervention: single (6 ponds) and multiple stocking of *G. affinis* (6 ponds) Control: 6 ponds Canals Intervention: *G.* | Estimated marginal mean values of younger (L1 and L2) and older (L3 and L4) *An. gambiae* s.l. larvae up to 13 weeks' follow-up | No difference between control and intervention groups at follow-up, apart from the numbers of older larvae were lower in the canal intervention group |

**Table 5. Summary of included studies** (*Continued*)

| | | | | |
|---|---|---|---|---|
| | | *affinis* (6 canals)<br>Control: 6 canals | | |
| | Mahmoud 1985 | Intervention: 20 canals treated with *G. holbrooki*<br>Control: 5 canals | Density of a late larval stage of *An. arabiensis* (L4) up to 13 months' follow-up | *An. arabiensis* density was lower in intervention canals for 2 months (5 months' and 6 months' post-intervention) just before and at the beginning of the dry season. Larval densities dropped in both intervention and control groups in the dry season (7 months' post-intervention) and at the end of the rainy season (13 months' post-intervention). Fish numbers did not increase after the rainy season and during the last 6 months of the study. According to the authors, control of the flow of water from large to branch canals by gates deprived the fish of free movement. In addition, during the rainy season, rainwater pools act as suitable larval habitats for *An. arabiensis*. |

# APPENDICES

## Appendix 1. Search methods: detailed search strategies

| Search set | CIDG SR[a] | CENTRAL | MEDLINE | Embase | LILACS | CAB ABSTRACTS |
|---|---|---|---|---|---|---|
| 1 | mosquito* | mosquito* | mosquito* | mosquito$ | mosquito$ | mosquito* |
| 2 | control* OR breeding* OR larva* Or predat* | control* OR breeding* OR larva* OR predat* | control* OR breeding* OR larva* OR predat* | control$ OR breeding$ OR larva$ Or predat$ | control$ OR breeding$ OR larva$ OR predat$ | control* OR breeding* OR larva* Or predat* |
| 3 | 1 and 2 | 1 and 2 | PEST CONTROL, BIOLOGICAL | VECTOR CONTROL | 1 and 2 | 1 and 2 |
| 4 | (fish* or frog*) | MOSQUITO CONTROL/ METHODS | 2 OR 3 | 2 OR 3 | (fish$ OR frog$) | (fish* or frog*) |
| 5 | larvivorous | 3 or 4 | 1 AND 4 | 1 AND 4 | larvivorous | larvivorous |
| 6 | 4 or 5 | (fish* OR frog*) | MOSQUITO CONTROL/ METHODS | (fish$ OR frog$) | 4 or 5 | "Gambusia" OR "Poecilia" OR "Aphanius" OR "Oreochromis" OR "Tilapia" OR "Aplocheilus" OR "Cyprimus" OR "Ctenopharyngodon" OR "Rasbora" OR "Aphyocypris" |
| 7 | 3 and 6 | larvivorous | 5 OR 6 | larvivorous | 3 and 6 | 4 or 5 or 6 |
| 8 | - | 6 OR 7 | (fish* OR frog*) | "Gambusia" OR "Poecilia" OR "Aphanius" OR "Oreochromis" OR "Tilapia" OR "Aplocheilus" OR "Cyprimus" OR "Ctenopharyngodon" OR "Ras- | - | 3 and 7 |

(*Continued*)

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | bora" OR "Aphyocypris" | | |
| 9 | - | 5 and 8 | larvivorous | 6 or 7 or 8 | - | - |
| 10 | - | - | "Gambusia" OR "Poecilia" OR "Aphanius" OR "Oreochromis" OR "Tilapia" OR "Aplocheilus" OR "Cyprimus" OR "Ctenopharyngodon" OR "Rasbora" OR "Aphyocypris" | 5 and 9 | - | - |
| 11 | - | - | 8 OR 9 OR 10 | - | - | - |
| 12 | - | - | 7 AND 11 | - | - | - |

[a] Cochrane Infectious Diseases Group Specialized Register.

## Appendix 2. Descriptive analysis of included studies

None of the included studies reported on cases of malaria, EIR, or the density of adult vector mosquitoes. Therefore, we did not find any direct evidence that this intervention impacts malaria transmission. We performed a descriptive analysis of the 14 included studies that examined the effect of fish stocking on immature anopheline mosquito presence or density, or both. We analysed the studies by the habitat type that study authors introduced for the larvivorous fish. Nine studies evaluated larvivorous fish in localized water bodies (including wells, domestic water containers, fishponds and pools, and riverbed pools created after dam construction), four studies used rice field plots, and two studies used water canals; see Table 2.

### Section 1: localized water bodies

#### Wells

Two studies from India evaluated larviciding in wells (Sitaraman 1976; Menon 1978).

Sitaraman and colleagues introduced fish (100 *Poecilia reticulata*) to 10 wells and maintained four wells as controls. The authors measured *An. stephensi* larval and pupal densities by taking five dips per well every four days until 28 days' post-intervention. They measured baseline values immediately before the introduction of larvivorous fish to the 10 wells. We examined the raw data reported by the authors for evidence of an effect of larvivorous fish on the immature *An. stephensi* population.

Baseline values in the control (four wells) and intervention groups (10 wells) were comparable before fish were introduced (assuming that these were the numerical totals across the 10 intervention and four control wells; **Table 1a**). In the intervention wells, immature mosquito numbers decreased rapidly after fish were introduced. This decrease in immature mosquito numbers was greater than in the control group. The study authors did not detect any immature mosquitoes in the 10 wells at four days' follow-up. They measured only 15 larvae at 24 days' post-intervention and 40 larvae at 28 days' post-intervention. At 28 days, the immature mosquito numbers (L1 to L4 stages) increased, and the study authors introduced fish into the control wells.

Sitaraman and colleagues released 50 fish per well into 12 wells, with five wells in the same ward serving as controls, and followed immature mosquito numbers for 22 days (**Table 2a**). A dramatic drop in larvae from daily dips (50 per well) was seen early, with a 69% reduction in larvae and an 82% reduction in pupae by day 2; no such change was seen in the control wells. However, recovery of relatively immature larvae (L1 and L2 instars) was relatively rapid and baseline values were restored by day 10; although recovery of mature larvae (L3 and L4) was slower and less complete, with mean density still 60% lower than baseline after three weeks (Table 1, page 317 of the paper).

*With high fish stocking levels, larvae are eliminated in the first four days in wells but reappeared at lower levels from day 24 onwards. With lower stocking levels, there was a partial effect for two weeks only, with rebound.*

**Table 1a.** Sitaraman 1976**: *An. stephensi immature numbers before and after introduction of fish (100 guppies per well)***

| **Intervention** | **Immature stages** | **Pre-intervention** | **Follow-up (days)** | | |
|---|---|---|---|---|---|
| | | | **4** | **24** | **28** |
| Control (4 wells) | L1 + L2<br>L3 + L4<br>Pupae | 296<br>346<br>44 | 236<br>254<br>64 | 94<br>36<br>24 | 240<br>156<br>16 |
| Intervention (10 wells) | L1 + L2<br>L3 + L4<br>Pupae | 890<br>960<br>205 | 0<br>0<br>0 | 15<br>0<br>0 | 40<br>0<br>0 |

**Table 2a.** Sitaraman 1976**: *An. stephensi immature numbers before and after introduction of fish (50 guppies per well)***

| **Intervention** | **Immature stages** | **Pre-intervention** | **Follow-up (days)** | | |
|---|---|---|---|---|---|
| | | | **4** | **16** | **22** |
| Control (5 wells) | L1 + L2<br>L3 + L4<br>Pupae | 275<br>330<br>40 | 455<br>255<br>40 | 525<br>245<br>30 | 300<br>255<br>40 |
| Intervention (12 wells) | L1 + L2<br>L3 + L4<br>Pupae | 384<br>546<br>102 | 156<br>156<br>84 | 498<br>204<br>42 | 486<br>222<br>48 |

In a second study from India, Menon and colleagues introduced *Gambusia* or *Aplocheilus* fish to 3438 wells but kept 317 wells as controls. In intervention sites, if they found mosquito larvae, they stocked with 50 fish per well; if no larvae were present, they stocked with 20 fish per well. They measured *An. stephensi* larval density at baseline and monthly for four months.

The proportion of wells with larvae was greater in the intervention group (32.8%) than in the control group (7.7%) at baseline (**Table 3a**). At follow-up, the proportion of wells with larvae dropped markedly in the intervention arm (less than 1%) but not in the control arm. In the control group, percentage of wells with larvae increased to a maximum of 9.6% during follow-up.

*This study appeared to provide evidence of a larvicidal effect of fish in wells using relatively high stocking levels.*

**Table 3a.** Menon 1978**: percentage of wells with *An. stephensi larvae in wells immediately before and after introduction of fish***

| Intervention | Pre-intervention (%) | Follow-up (months) | | |
|---|---|---|---|---|
| | | 1 | 2 | 4 |
| Control | 7.7 | 8.0 | 8.6 | 9.6 |
| Intervention | 32.8 | 0.97 | 0.49 | 0.47 |

#### Domestic water containers

Three studies examined larviciding in domestic water containers (Fletcher 1992; Haq 2013; Sabatinelli 1991). In Ethiopia, Fletcher and colleagues introduced fish to wells, barrels, cisterns, and washbasins. In Gujarat State, India, Haq and colleagues added fish to water storage containers, such as cement tanks including underground tanks, kothi (big mud pots), and barrels. On the Comoro Islands, located off the south-east coast of Africa, Sabatinelli and colleagues introduced fish to ablution basins and tanks.

Fletcher 1992 introduced *Aphanius dispar* to 60 domestic water containers and kept 51 water containers as controls. They measured the *Anopheles culicifacies adanensis* larval population using a standard dipping procedure pre-intervention and then either every two weeks (May to August 1987) or monthly for 11 months. Control and intervention values were identical at baseline (0%). Sites allocated to the fish intervention had fewer *An. culicifacies adanensis* larvae at one year post-intervention compared with control sites (see **Table 4a**). *Fish introduction appears to prevent an increase in the number of domestic water container sites with larvae compared with controls up to 11 months' follow-up.*

**Table 4a.** Fletcher 1992**: percentage of sites with *An. culicifacies adanensis larvae before and after introduction of fish***

| Intervention | Pre-intervention (percentage of sites) | Follow-up (months) | | | |
|---|---|---|---|---|---|
| | | 1 | 4 | 7 | 11 |
| Control | 0 | 0 | 2.0 | 13.7 | 4.2 |
| Intervention | 0 | 0 | 0.9 | 0 | 0 |

Haq 2013 added *A. dispar* to 295 water containers in the intervention village and monitored 30 of these containers, and monitored 25 containers in the control village. The study authors measured the *An. stephensi* larval and pupal population from trial initiation in December 2010 for 12 months (up to November 2011) using a standard dipping procedure taking the mean of three dips at weekly intervals for four weeks followed by fortnightly. The study authors only reported data as % reduction in immature density of L3 and L4 larvae plus pupae of *An. stephensi* at day 0, 7, 15, and every 15 days thereafter. The percentage reduction was greater than 60% for all time points, even up to one year post-intervention (see **Table 5a**).

**Table 5a:** Haq 2013**: percentage reduction in L3-L4 larvae and pupae of *An. stephensi after introduction of fish***

| Outcome | Follow-up (months) | | | | |
|---|---|---|---|---|---|
| | Baseline | 1 | 4 | 7 | 12 |
| **% reduction in *An. stephensi L3-L4 larvae and pupae*** | 0 | 94.39 | 97.14 | 100 | 96.08 |

Sabatinelli 1991 introduced *P. reticulata* to domestic water containers in Hantsamboa village (39 ablution basins sites in November 1987, total number of sites not specified) and kept 20 ablution basins in Bandamadji village as control sites. They measured the

percentage of containers positive for *An. gambiae* larvae by examining the surface and bottom of containers (at least 15 cm in diameter) in both intervention and control groups four times during the 11 months' follow-up. Control and intervention values were identical at baseline. At follow-up, the proportion of sites positive for *An. gambiae* larvae decreased at fish-treated sites but not at control sites (see **Table 6a**).

*This study appeared to show fish that reduce the number of domestic wash basins with larvae when added to these sites for up to 11 months.*

**Table 6a.** Sabatinelli 1991**: percentage of sites with *An. gambiae larvae before and after introduction of fish***

| Intervention | Pre-intervention (% of sites) | Follow-up (months) | | |
|---|---|---|---|---|
| | | 1 | 5 | 11 |
| Control | 40 | 75 | 45 | 50 |
| Intervention | 41 | 7 | 1 | 8 |

#### Fishponds and pools

Two studies based in Kenya examined the use of larvivorous fish in ponds (Howard 2007; Imbahale 2011a).

Howard and colleagues compared two intervention ponds and one control pond, all located within 150 m of each other. They measured the number of immature *An. gambiae* and *An. funestus* mosquitoes by taking larval dips five to seven days per week. We explored the evidence for an effect, if any, in three ways: we made a simple description of trends in the graph; we extracted data carefully from the graph; and we examined the authors' analysis.

**Trends in the graph:** the authors provided a detailed graph showing *An. gambiae* immature populations over time in the three ponds. They used a 15-week baseline period before the fish were introduced into two of the three ponds. The control pond had much lower densities of *An. gambiae* immatures in the baseline period, with none present in the first 1.5 months; then followed a gradual increase in density month by month over the intervention period, with wide week-by-week and, at certain time points day-by-day, variations. At six months' post-intervention, larvae numbers peaked and the authors introduced fish to the control pond.

For the first intervention pond, densities were much higher than for the control pond at baseline. When fish were introduced, the density remained low, or possibly attenuated. For the second intervention pond, the intervention did not appear to be associated with any substantive visual pattern of reduction in density, although it could be argued that some attenuation was evident in the first five months. Thus, critical appraisal of Figure 2 in Howard 2007 indicated increasing immatures in the control pond but did not provide convincing evidence of substantial and sustained decline in the two intervention ponds.

**Extracting data from the graph:** we took fixed time points before and after the intervention. **Table 7a** shows these data, which we estimated using a ruler against the y axis. We chose the one- and three-month time points as standard normal values. We did not include the end time point of the experiment - when the study authors introduced fish to the control pond - as this will introduce bias as it is defined by an increase in larvae. Our analysis below supported evidence of reduction in the immature *An. gambiae* population in the first intervention pond but not in the second intervention pond.

**Table 7a.** Howard 2007**: *An. gambiae immatures in three ponds before and after the introduction of fish***

| Intervention | Pre-intervention (months) | | Follow-up (months) | |
|---|---|---|---|---|
| | 3 | 1 | 1 | 3 |
| Control pond | 0 | 7 | 7 | 4 |
| First intervention pond[1] | 3 | 7 | 0 | 0 |

(*Continued*)

| | | | | |
|---|---|---|---|---|
| Second intervention pond[2] | 2 | 4 | 2 | 2 |

[1]Referred to as pond C within Howard 2007 study.
[2]Referred to as pond D within Howard 2007 study.

**Authors' analysis:** the authors used Mulla's formula to calculate percentage reduction in *An. gambiae* and *An. funestus* immatures, with estimates of 95.8% reduction in *An. gambiae* immatures in intervention pond 1 and 94.1% for intervention pond 2; and similar high reductions for *An. funestus* (98.3% in intervention pond 1, 97.5% in intervention pond 2). However, Mulla's formula depends on rates in the control arm, in which an increase in immature numbers was clearly seen over time. Therefore, one interpretation of these data is that fish are effective; the other is that these large effects are the result of particular ecological changes happening in the control pond.
*This study appeared to provide limited evidence of a possible larvicidal effect of fish in ponds.*
For the Imbahale 2011a study, refer to the water canals section below.

#### Riverbed pools below dams

Two studies in Sri Lanka evaluated fish introduced to riverbeds pools located below dams (Kusumawathie 2008a; Kusumawathie 2008b).
In the Kusumawathie 2008a study, authors introduced *P. reticulata* to 29 riverbed pools below Kotmale dam and used 31 pools as controls. They measured the number of immature *Anopheles* using a 100 mL larval dipper at a frequency of six dips per m² at baseline (day before fish were introduced) and up to 120 days' follow-up. Control and intervention groups had similar baseline values. At follow-up, the intervention group had greater reductions than the control group for the outcomes of percentage of pools with *Anopheles* larvae, mean number of larvae per pool, and mean number of larvae per 100 dips (**Table 8a**).
*This study appears to provide evidence of a larvicidal effect of fish in riverbed pools below dams sustained up to four months.*
**Table 8a. Kusumawathie 2008a: mean percentage of pools with *Anopheles larvae, mean number of larvae per pool, and mean number of larvae per 100 dips before and after introduction of larvivorous fish***

| Outcome | Intervention | Pre-intervention | Follow-up |
|---|---|---|---|
| Percentage of pools with *Anopheles* larvae | Control<br>Intervention | 100<br>100 | 31.03<br>0 |
| Mean number of larvae per pool | Control<br>Intervention | 3.03<br>3.17 | 0.52<br>0 |
| Mean number of larvae per 100 dips | Control<br>Intervention | 114.63<br>109.52 | 20<br>0 |

In the second study (Kusumawathie 2008b), Kusumawathie and colleagues introduced *P. reticulata* to all riverbed pools in Laxapana and Kotmale 1 study sites. They used riverbed pools in Kotmale 2 and Nilambe as control sites. They measured immature *Anopheles* densities using a 100 mL larval dipper at a frequency of six dips per m² for one year pre-intervention and one year post-intervention. Baseline values at control and intervention sites were similar for the outcomes percentage pools with *Anopheles* larvae and mean number of larvae per 100 dips, but not for mean number of larvae per 100 pools. At follow-up, the riverbed pools stocked with fish had larger reductions in terms of presence and density of larvae (**Table 9a**).
*This study indicated a partial effect of fish on presence and density of larvae in riverbed pools below dams for up to one year.*
**Table 9a. Kusumawathie 2008b: mean percentage of pools with *Anopheles larvae, mean number of larvae per 100 pools, and mean number of larvae per 100 dips before and after introduction of larvivorous fish***

| Outcome | Intervention | Pre-intervention | Follow-up |
|---|---|---|---|
| Percentage of pools with *Anopheles* larvae | Control<br>Intervention | 15.95<br>17.39 | 12.52<br>5.79 |
| Mean number of larvae per 100 pools | Control<br>Intervention | 28.78<br>142.94 | 27.44<br>11.25 |
| Mean number of larvae per 100 dips | Control<br>Intervention | 8.52<br>11.84 | 9.02<br>3.4 |

### Section 2: rice field plots

Five studies evaluated fish introduced to rice fields: one in Central Java (Nalim 1988), two in South Korea (Kim 2002; Yu 1989), and two in Tajikistan (RTDC 2008; Zvantsov 2008).

In Central Java, Nalim and colleagues stocked 23.9 ha of rice fields with *P. reticulata* and *Cyprinus carpio* fish. They did not specify the size of the control area or the total number of control and intervention field plots. Using 80 emergence traps randomly located in the treated and control areas, they reported the numbers of *Anopheles aconitus*, *Anopheles barbirostris*, and *Anopheles annularis* newly emerged adult mosquitoes collected/m²/day (trap area = 0.25 m²) over six years. Effects were mixed, with some evidence of an impact of fish on *An. aconitus* and *An. annularis*, but not on *An. barbirostris* (**Table 10a**).

*This study indicates a partial effect of fish on the density of newly emerged An. aconitus and An. annularis, but not An. barbirostris, in rice field plots below dams for up to six years.*

**Table 10a.** Nalim 1988**: mean number of adult mosquitoes collected per m²per day**

| Species | Intervention | Year | | |
|---|---|---|---|---|
| | | 1 | 3 | 6 |
| *An. aconitus* [1] | Control<br>Intervention | 2.4<br>3.35 | 4.2<br>0.2 | 1.2<br>0.01 |
| *An. barbirostris*[1] | Control<br>Intervention | 7.6<br>6.0 | 6.0<br>4.7 | 3.2<br>2.9 |
| *An. annularis* [1] | Control<br>Intervention | 3.0<br>3.35 | 4.2<br>1.13 | 2.2<br>0.7 |

[1]We discarded two years of data (1982, 1983), as the study authors reported that the control area was sprayed with fenitrothion (a phosphorothioate (organophosphate) insecticide) at the end of 1982.

In the South Korean study, Kim and colleagues introduced three slightly different interventions to three rice field plots measuring about 300 m² to 600 m² (Kim 2002). They compared these with a control area of three rice field plots of similar size. They introduced either *Tilapia mossambicus* and *Aplocheilus latipes* (treatment A) or *Aphyocypris chinensis* and *Tilapia mossambicus* (treatment B and treatment C) to rice field plots and took two dips, with between two and four replicates per rice field, every two weeks, to examine the mean number of *An. sinensis* larvae.

We extracted data for specific time points before and after the intervention. The study authors used a six-week baseline period for treatments B and C but no baseline for treatment A before the fish were introduced into two plots.

The results provided a robust controlled before-and-after study (treatments B and C), with four time points in the control period (**Table 11a**). Baseline measurements appeared similar at control and intervention sites. In the control group and for treatments B and C, the

number of *An. sinensis* larvae was higher at two weeks' pre-intervention than at six weeks' pre-intervention. After fish were introduced to the intervention sites, the *An. sinensis* larval population in the control group was the same at two weeks' follow-up but decreased at six weeks' follow-up. Larvae were clearly reduced at the two sites where fish were introduced.

The study also afforded a controlled time series comparison between the control group and a third intervention site, where the fish were introduced at the start of observations (treatment A; **Table 12a**). The number of *An. sinensis* larvae increased between one week' and five weeks' follow-up at both control and intervention sites. However, the number of larvae decreased by 13 weeks' follow-up at both control and intervention sites. This shows a mean difference in larvae density between control and intervention over the entire period of observation. However, these data were weaker, as no baseline density was noted in the intervention arm, and any difference from the control could be due to chance.

*This study appeared to provide limited evidence of a possible larvicidal effect of fish on An. sinensis larvae in rice field plots.*

**Table 11a**. Kim 2002: ***An. sinensis*** **larvae at control (three plots) and intervention sites (two plots) before and after introduction of fish**

| Intervention | Pre-intervention (weeks) | | Follow-up (weeks) | |
|---|---|---|---|---|
| | 6 | 2 | 2 | 6 |
| Control | 2.0 | 4.5 | 4.5 | 2.5 |
| Treatment B | 2.5 | 3.5 | 2.25 | 0.4 |
| Treatment C | 1.75 | 4.13 | 2.25 | 0.38 |

**Table 12a.** Kim 2002: ***An. sinensis*** **larvae at control plots (three plots) and at an intervention plot (one plot) after introduction of fish**

| Intervention | Follow-up (weeks) | | | |
|---|---|---|---|---|
| | 1 | 5 | 9 | 13 |
| Control | 2.0 | 4.5 | 4.5 | 2.5 |
| Treatment A | 1.25 | 2.5 | 2.0 | 0.5 |

In South Korea, Yu and colleagues compared ponds treated with two species of fish (*A. latipes* and *Tilapia mossambicus)*, one species alone (*A. latipes*), and a control group (Yu 1989). The researchers selected six plots, 45 m² in size and 0.3 m in depth, located within a confined rice field of 1000 m². They randomly assigned two plots to each treatment group. They took measurements of the *An. sinensis* larval population every week, using a 500 mL dipper (two to four dips per rice field plot) or a nylon net (eight to 10 sweepings per sample).

The study authors monitored the *An. sinensis* larval population for eight weeks before they introduced fish, and pre-intervention values were comparable between sites. In the first two intervention plots, they introduced one fish species: at four weeks, larvae had increased against baseline in both control and intervention ponds, but the size of the increase was smaller in the one-fish intervention pond (7.00 compared with 16.00, 56% lower; **Table 13a**).

In the next two intervention plots, they introduced two fish species, and follow-up at four and seven weeks showed considerably lower values in the two-fish intervention pond than in the control pond (4.21 compared with 16.13, 74% lower; **Table 13a**).

*This study provided some evidence that larvivorous fish can constrain the rapid increases in larvae populations seen in untreated ponds.*

**Table 13a.** Yu 1989: **mean number of** ***An. sinensis larvae in ponds before intervention and after introduction of fish***

| Intervention | Pre-intervention[1] | Follow-up (weeks) | |
|---|---|---|---|
| | | 4 | 7 |
| Control | 4.56 | 16.0 | 16.13 |
| 1 fish species | 4.19 | 7.00 | Bacteria introduced |
| 2 fish species | 4.50 | 4.87 | 4.21 |

[1]We recalculated the mean pre-intervention values that the study authors reported in control and intervention groups, as the study authors incorrectly reported these values.

In Tajikistan, RTDC 2008 compared two rice field sites treated with *Gambusia affinis* compared with two control sites. The study authors did not state the size of the plots and how treatment was assigned to the sites. They sampled the immature *Anopheles* population and *G. affinis* population every 10 or 11 days using a standard net of 20 cm diameter, which was held to 0.5 m in one direction and then taken in the opposite direction. The net contents were rinsed and the number of fish, and mosquito larvae and pupae counted. Five such samples gave the number of fish and the immature mosquitoes/m².

The study authors did not report any baseline data. They monitored the immature *Anopheles* population up to either 41 (Vakhsh district site Kirov 2) or 40 days (Bokhta district site of Saidov (**Table 14a**). The number of immature *Anopheles* mosquitoes were comparable in both control sites over this time period, and a decrease in the number of immature of *Anopheles* mosquitoes was observed in the intervention sites.

*This study appeared to provide limited evidence of a larvicidal effect of G. affinis fish on immature* Anopheles*mosquitoes in rice field plots.*

**Table 14a.** RTDC 2008**: number of immature *Anopheles mosquitoes after introduction of fish***

| Intervention | Follow-up (days) | | | | |
|---|---|---|---|---|---|
| | 0 | 10 | 20/21 | 30/31 | 40/41 |
| Control (Vakhsh district) | 30 | 38 | 40 | 41 | 43 |
| Intervention (Vakhsh district) | 36 | 20 | 8 | 3 | 2 |
| Control (Bokhta district) | 34 | 38 | 38 | 41 | 44 |
| Intervention (Bokhta district) | 42 | 25 | 6 | 3 | 1 |

The authors of Zvantsov 2008 examined the effect of introduction of either *G. affinis* or *P. reticulata* to rice field plots in two districts with established rice production: Farkhor (Kizilpakhtachi village) and Shaartuz (Birlyash village). In each rice field nine checks were used: three filled with *P. reticulata*, three filled with *G. affinis*, and three served as controls. For this study, each rice check measured 3 m × 3 m, and checks treated with the different species of fish were arranged in a chequer board pattern. Fish were released into the rice checks at the rate of five pregnant females/m², i.e. 45 females per 3 m × 3 m check at day 0. Fish were restocked at day 38 or 39 in both sites, but it is unclear whether *P. reticulata* alone was restocked due to poor survival or both *P. reticulata* and *G. affinis* were restocked. The study authors reported the mean value of younger larvae and of older larvae per m².

Mean baseline data, measured immediately before introduction of fish, was comparable in the control and intervention checks in Shaartuz (Birlyash village). In Farkhor district (Kizilpakhtachi village), mean baseline values reported were lower in the control checks than in the intervention checks. The study authors monitored the immature *Anopheles* population up to 62 (Birlyash village) or 65 days (Kizilpakhtachi village) after introduction of fish. The number of immature *Anopheles* mosquitoes decreased in both intervention sites using *G. affinis* and in one site using *P. reticulata* (Kizilpakhtachi village) (**Table 15a; Table 16a)**. However, in Shaartuz district (Birlyash village), a partial effect was noted using *P. reticulata* with rebound above baseline levels (**Table 15a**).

*This study provided some evidence of a larvicidal effect of G. affinis fish on immature Anopheles mosquitoes in rice field plots. With P. reticulata, there was some evidence of a larvicidal effect in one district and a partial effect in one district with rebound.*

**Table 15a.** Zvantsov 2008**: density of younger and older anopheline larvae after introduction of fish in Shaartuz district (Birlyash village)**

| Intervention | Larvae | Follow-up (days) | | | | |
|---|---|---|---|---|---|---|
| | | **0** | **10** | **33** | **48** | **62** |
| Control | Younger | 6 | 8 | 17 | 28 | 30 |
| | Older | 4 | 3 | 7 | 12 | 14 |
| *G. affinis* | Younger | 8 | 3 | 3 | 1 | 0 |
| | Older | 5 | 2 | 0 | 0 | 0 |
| *P. reticulata* | Younger | 7 | 5 | 15 | 16 | 20 |
| | Older | 4 | 1 | 4 | 5 | 10 |

**Table 16a.** Zvantsov 2008**: density of younger and older anopheline larvae after introduction of fish in Farkhor district (Kizilpakhtachi village)**

| Intervention | Larvae | Follow-up (days) | | | | |
|---|---|---|---|---|---|---|
| | | **0** | **14** | **29** | **44** | **65** |
| Control | Younger | 3 | 10 | 11 | 16 | 17 |
| | Older | 1 | 2 | 7 | 4 | 7 |
| *G. affinis* | Younger | 9 | 2 | 7 | 0 | 2 |
| | Older | 6 | 1 | 2 | 0 | 0 |
| *P. reticulata* | Younger | 7 | 1 | 7 | 0 | 2 |
| | Older | 4 | 1 | 2 | 0 | 2 |

### Section 3: water canals

Two studies introduced fish to irrigation canals - one in Kenya (Imbahale 2011a) and one in Sudan (Mahmoud 1985).

In Kenya, Imbahale and colleagues compared the effects of *G. affinis* introduced to ponds or water canals versus control sites. The water sources were discrete; 18 ponds were 1 m² in size and 1 m depth, and 12 canals were 15 m² in size and 0.3 m in depth. For ponds, the authors evaluated the effects of single stocking and multiple stocking of fish by measuring *An. gambiae* s. l. larvae twice a week for 13 weeks; and for canals, they compared controls with a single stocking of fish. The study authors divided outcomes by younger larvae (L1 and L2) and older larvae (L3 and L4), and reported estimated marginal mean values. No difference was demonstrated between control and intervention groups at follow-up, apart from the fact that the numbers of older larvae were smaller in the canal intervention group (**Table 17a**).

*This study provided some evidence of an effect of larvivorous fish up to 13 weeks in water canals but not in ponds.*

**Table 17a.** Imbahale 2011a: **estimated marginal mean values of immature anopheline numbers after introduction of fish**

| **Intervention** | | **Follow-up** | |
|---|---|---|---|
| | | **Younger larvae (L1 and L2)**[1] | **Older larvae (L3 and L4)**[1] |
| Ponds | Control | 2.667 (2.217 to 3.117) | 0.758 (0.551 to 0.964) |
| | Fish (stocked once) | 2.667 (2.217 to 3.117) | 0.964 (0.757 to 1.170) |
| | Fish (multiple stocking) | 3.067 (2.604 to 3.505) | 0.903 (0.697 to 1.109) |
| Canal | Control | 3.417 (2.896 to 3.937) | 1.177 (0.974 to 1.380) |
| | Fish (stocked once) | 1.906 (1.386 to 2.427) | 0.547 (0.344 to 0.750) |

[1]The study authors reported the estimated marginal mean ± 95% confidence interval (CI).

In Sudan, Mahmoud and colleagues introduced *G. affinis* to Gezira irrigation canals (4 km to 10 km in length, 2 m in width, 1 m in depth). They used 20 canals in the intervention group and five canals in the control group. In intervention canals, they released fish at 1 km intervals. They measured the density of a late larval stage of *Anopheles arabiensis* (L4) larvae in both intervention and control canals by performing larval dips at two spots per kilometre in each canal, reporting means by month from weekly dipping of 10 dips per spot for 14 months.

No baseline was provided, but *An. arabiensis* density was less in intervention canals for two months (five months' and six months' post-intervention) just before and at the beginning of the dry season (**Table 18a**). Larval densities dropped in both intervention and control groups in the dry season (seven months' post-intervention) and at the end of the rainy season (13 months' post-intervention). Fish numbers failed to increase after the rainy season and during the last six months of the study. According to the authors, control of the flow of water from large to branch canals by gates deprived the fish of free movement. Also, during the rainy season, rainwater pools act as suitable larval sites for *An. arabiensis*.

*Introducing larvivorous fish appeared to partly constrain An. arabiensis larval density increases at the beginning of the dry season.*

**Table 18a.** Mahmoud 1985:**density of *An. arabiensis L4 larvae after introduction of fish***

| **Intervention** | **Follow-up (months)** | | | |
|---|---|---|---|---|
| | **3** | **5** | **7** | **13** |
| Control | 42 | 153 | 7 | 125 |
| Intervention | 25 | 24 | 1 | 124 |

## WHAT'S NEW

| Date | Event | Description |
|---|---|---|
| 7 December 2017 | New search has been performed | We updated the literature search to 6 July 2017 and included three new studies that reported only outcomes relevant to our secondary analysis. The conclusions remain the same as the last published version (Walshe 2013). |
| 7 December 2017 | New citation required but conclusions have not changed | This is an update of a Cochrane Review published in 2013. |

## CONTRIBUTIONS OF AUTHORS

TB and PG conceived the review and wrote the protocol, with input from Robert A Wirtz (previous author), Raymond Beach (previous author), GHP, and AAA.

DPW, AAA, GP, PG, and TB screened articles.

TB, AAA, PG, and DPW extracted data from the included studies.

DPW constructed the tables, prepared the GRADE summaries, and wrote the review.

PG helped with determining study inclusion, planning how to construct the review, summarizing the data, GRADE assessments, and editing the review.

All review authors read and approved the final manuscript.

## DECLARATIONS OF INTEREST

DPW is supported by the Effective Health Care Research Consortium. This Consortium is funded by UK aid from the UK Government for the benefit of low- and middle-income countries (Grant: 5242). DPW acted as rapporteur between 2011 and 2014 for the Innovative Vector Control Consortium (IVCC) at their External Scientific Advisory Committee (ESAC) meetings.

TB was on the Global Fund Technical Review Panel as well as the Vector Control Advisory Group as a non-paid advisor and presently serves on the WHO Malaria Policy Advisory Committee (MPAC).

PG is Director of the Evidence Building and Synthesis Research Consortium, which receives money to increase the number of evidence-informed decisions by intermediary organizations, including World Health Organization (WHO) and national decision makers, that benefit the poor in low- and middle-income countries. PG is the co-ordinator of a WHO Collaborating Centre for Evidence Synthesis for Infectious and Tropical Diseases (apps.who.int/whocc/default.aspx; UNK234): one of the Centre's aims is to help WHO in its role as an infomediary in communicating reliable summaries of research evidence to policy makers, clinicians, teachers, and the public in low- and middle-income countries.

AAA presently serves on the WHO MPAC.

GP carries out research on frogs, including impacts arising from introduced *Gambusia*. He is also interested in possible impacts of larvivorous fish on mosquito populations and malaria transmission, and has separately reviewed these issues, but has no relevant vested interests.

## SOURCES OF SUPPORT


### Internal sources

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- World Health Organization (WHO), Switzerland.

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## DIFFERENCES BETWEEN PROTOCOL AND REVIEW

DPW was added as author on the review. Robert A Wirtz and Raymond Beach stepped down as authors on the review. We added EIR as an outcome, as an effect demonstrated on this would be an extremely useful indicator of an effect on malaria transmission. We limited inclusion of studies monitoring secondary outcomes to studies with a follow-up period longer than three weeks after introduction of larvivorous fish.

### Differences between review and review update

We amended Ahmed A Abdel-Hameed Adeel to Ahmed A Adeel, and Tom Burkot to Thomas R Burkot.

## INDEX TERMS

### Medical Subject Headings (MeSH)

*Anopheles [parasitology]; *Disease Vectors; *Feeding Behavior; *Fishes; Disease Reservoirs [parasitology]; Larva; Malaria [*prevention & control; transmission]; Mosquito Control [*methods]; Plasmodium

### MeSH check words

Animals